Multi-scale Investigation of Weight-bearing Exercise on Bone Biomechanical Integrity in the Osteogenesis Imperfecta Model (*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[1]. The *oim/oim* mice, homozygous for a null mutation in the COL1A2 gene of type I collagen, have significantly reduced bone biomechanical integrity as well as altered bone mineral composition[2,3]. Heterozygous mice (+/*oim*) have a phenotype intermediate to *oim/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 26% of their peak bone mass during the normal 2 year prepubertal/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 (muscle 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 success 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 Pathobiology (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 at 10 m/min (7° incline) 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 plantaris and gastrocnemius were exposed, tied off with 4-0 silk and attached to a Grass force transducer of the PowerLab™[4]. The sciatic nerve was placed on a bipolar stimulation electrode and maximal contractile tension-generating capacity [peak tetanic tension (P_o)] was elicited using ~ 7 volts, 150 Hz, 250 msec, 0.3 trains/sec. P_o values were normalized to cross sectional area to determine muscle quality.

Muscle Mass Determination and Histologic Analyses

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 cross-sectional muscle fiber areas was determined per muscle. Muscle fibers were also evaluated for evidence of pathology.

Bone Biomechanical Testing (Torsional Loading to Failure)

Right femora were excised upon sacrifice and cleaned of soft tissue. μCT analyses were performed (MicroCAT II) and the data reconstructed (Amira 3.1) prior to torsional loading to failure. A 5 kg load cell was used with a constant torsional force of 0.75 rad/sec (TA-HDi). The maximum force required to break the bone was determined by the computer controlling the test machine and used in conjunction with geometric data from μCT analysis to calculate whole bone stiffness, material shear modulus and tensile strength and strain energy to failure[5].

Fourier Transform Infrared (FTIR) and Scanning Acoustic Microscopy (SAM)[6]

3μm thick bone sections were used for μFTIR (Perkin Elmer Spectrum Spotlight FTIR chemical imaging system) and the remaining slabs for SAM (Kraemer Scientific Instruments). For SAM the bone was covered with H_2O or PBS and imaged using the Kraemer high and low frequency SAMs. The quantitative micro-mechanical properties were obtained by evaluating gray level variations in a SAM image, providing a clear depiction of the inhomogeneities and heterogeneities in the sample.

Contractile Generating Capacity of Plantaris and Gastrocnemius Muscles

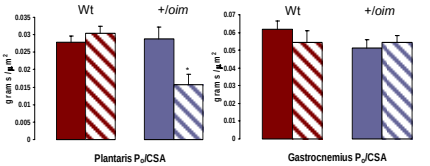

Figure 1. Specific Tension of Plantaris and Gastrocnemius Muscles of Non-exercised Control and Treadmill Exercised Wildtype (Wt) and Heterozygous (+/*oim*) Mice

Treadmill exercise significantly reduced specific tension of the plantaris in +/*oim* mice without impacting the gastrocnemius. No changes were seen in either muscle in response to treadmill exercise in Wt mice.
*p<0.05 compared to control
Solids: Controls; *Stripes*: Treadmill Exercise

Femoral Geometry and Torsional Loading to Failure

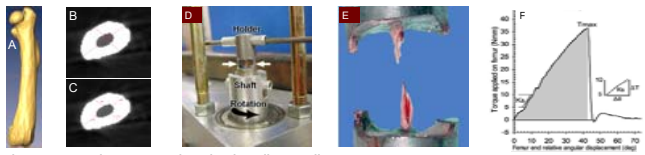

Figure 2. Femoral Geometry and Torsional Loading to Failure.
A) Reconstruction of μCT data to determine total femur length. Single slices are taken from the mid-shaft (B, C). D) The test fixture restrains the top of the femur holder while the bottom is axially rotated. Struts (*white* arrows) are cut prior to rotation. E) A typical post-test spiral fracture. F) 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

	Femur Length (mm)	Marrow Cavity Diameter (mm)	Cortical Bone Width (mm)	Polar Moment of Area (K; mm⁴)
Wt Control (n=14)	15.78±0.11	0.75±0.02	0.42±0.01	0.56±0.02
Wt Treadmill (n=13)	15.79±0.11	0.72±0.02	0.44±0.01	0.58±0.02
***+/oim* Control** (n=12-13)	16.07±0.12	0.67±0.02	0.44±0.01	0.49±0.01
***+/oim* Treadmill** (n=3)	15.59±0.09*	0.58±0.06	0.47±0.02	0.46±0.01

*p=0.056 compared to control

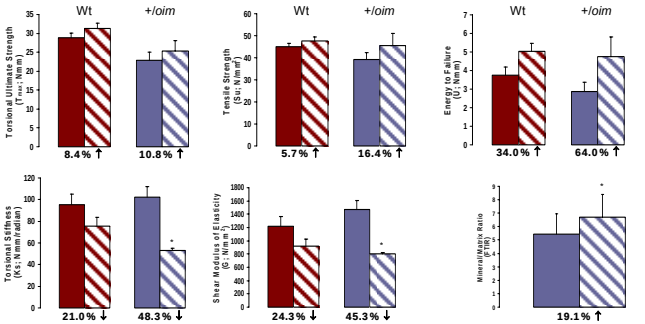

Figure 3. Femoral Strength and Stiffness of Non-exercised Control and Treadmill Exercised Wt and +/*oim* Mice
While not significant, treadmill exercise increased whole bone and material strength as well as energy to failure in both Wt and +/*oim* mice. Treadmill exercise decreased whole bone and material stiffness in both Wt and +/*oim*. +/*oim* mice appeared to have the greatest gains from treadmill exercise, possibly due to an increased mineral:matrix ratio following treadmill exercise.
*p<0.05 compared to control *Solids*: Controls; *Stripes*: Treadmill Exercise; *Red*: Wt; *Blue*: +/*oim*

Fourier Transform Infrared (FTIR) and Scanning Acoustic Microscopy (SAM) of Wildtype and *oim/oim* bone

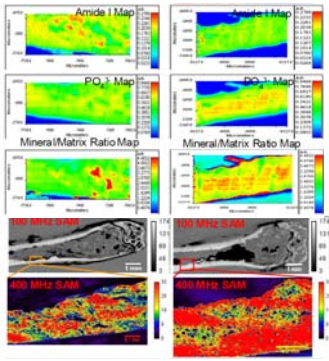

Figure 4. Fourier transform infrared (FTIR) microscopy and scanning acoustic microscopy (SAM) determination of a bone's structural/chemical/material properties of the same specimen region in Wt and *oim/oim* femora.

FTIR is a powerful tools for assessing the composition of mineralized tissues such as the content, composition and crystallinity of mineral, and the content, structure and quality of collagen. The results indicate that the collagen content (the amide I map), mineral phosphate content (the PO_4^{3-} map) and the mineral/matrix ratios are different between Wt and *oim/oim* bone. The collagen content in Wt bone is greater than in *oim/oim* bone.

SAM micrographs of a portion of 4-month femoral cortical bone cut parallel to the bone axis from the Wt (left) and *oim/oim* (right) mice. The images collected at different frequencies have different resolutions. The nominal lateral resolutions of 100 and 400 MHz SAM are 15 and 2.5 um, respectively. The 100 MHz images give the overall structure of bone sections, and the gray level variations are a result of the local changes in acoustic impedance (thus Young's modulus). The lamellar structure and osteocytes are more clearly visualized in 400 MHz images. The distribution of Young's modulus and overall values of *oim/oim* bone are slightly higher than those of Wt bone.

The combination of SAM and FTIR imaging has the potential to allow us to determine the molecular structure and micromechanical properties of the same region.

CONCLUSIONS

- Neither +/*oim* nor *oim/oim* plantaris or gastrocnemius muscles demonstrated any signs of necrosis, degeneration or regeneration in control or exercise animals (data not shown).
- +/*oim* mice were able to tolerate moderate treadmill exercise while *oim/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 exercise.
- FTIR analysis of Wt and *oim/oim* femora demonstrate differences in collagen content while SAM of the same femora indicated that the distribution of modulus and overall values are slightly higher in *oim/oim* femora as compared to Wt.

Summary

Taken together, these data indicate that +/*oim* mice are able to withstand moderate treadmill exercise and that this exercise has the potential to improve bone strength and reduce stiffness in +/*oim* mice with compromised bone, although further study is needed to elucidate the mechanisms behind this improvement.

By combining the use of μCT, torsional loading to failure and the unique capabilities of FTIR and SAM, we can perform bone whole mechanical and material property analyses with structural, chemical, and mechanical characterization over the same small region of the interfaces between apatite and matrix, and define how exercise deficiency will affect these properties.

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 *G610C 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.

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