Muscle Fiber-type Switching in Osteogenesis Imperfecta

Emily Harrelson[1], Victoria Gremminger[1], Cate Omosule[1], Charlotte Phillips[1,2]

Department of Biochemistry[1], Department of Child Health[2], University of Missouri-Columbia

Introduction

Osteogenesis imperfecta (OI) is a rare, genetic condition that exhibits brittle bones, muscle weakness, and short stature. OI is due to mutations in the type I collagen (COL1A1 and COL1A2) about 85% of the time. Osteogenesis imperfecta ranges in severity, including type I OI, which is mild, and type III, which is the most severe viable form of OI[1]. Our lab uses the osteogenesis imperfecta murine (*oim*) model. In the homozygote (*oim/oim*) form, the mice model a type III severe OI phenotype.

Muscle weakness in patients with OI has proven to be inherent. Increased muscle strength is associated with increased bone strength. Bone and muscle communicate through mechanotransduction, so when muscle exerts greater force on bone, the bone mass and bone strength increase. In addition to muscle weakness, mitochondria in the *oim/oim* mouse muscle have been shown to be dysfunctional[4]. Mitochondria provide energy to muscles. Type I myofibers are slow twitch and rely mostly on oxidative phosphorylation for energy, type IIa myofibers rely on oxidative phosphorylation and glycolysis, and type IIb fibers are fast twitch and rely mostly on glycolysis[5]. In this study, the slow-twitch soleus muscles of *oim/oim* mice and wild type mice were fiber-typed using immunohistochemical staining to determine if muscle fiber-type switching was occurring in *oim/oim* mice.

Materials and Methods

Mice were euthanized humanely, and muscles were harvested and were placed into optimal cutting temperature (OCT) compound which was frozen with liquid nitrogen cooled 2-methyl butane. The muscles were stored at -80°C and cut using a cryostat into 12 µm sections. Primary anti-bodies conjugated staining myosin heavy chains were mouse IgG2b BA.D5 (1:100), mouse IgG1 SC.71 (1:200), and mouse IgM BF.F3 (1:100) (DHSB, Iowa) for type I, type IIa, and type IIb, respectively. Rabbit IgG anti-laminin antibody (1:200) (ab11575; abcam) were used to stain the muscle fiber border. Secondary Alexa Fluor antibodies used were 647 goat anti-mouse IgG2b (1:250), 488 goat anti-mouse IgG1 (1:500), 555 goat anti-mouse IgM (1:500), and 350 goat anti-rabbit IgG (1:400) (Invitrogen, USA). Images were captured with the Zeiss Axiovert 200M with fluorescence and ORCA-ER camera and image analysis was performed with Fiji.

Myofiber Immunohistology

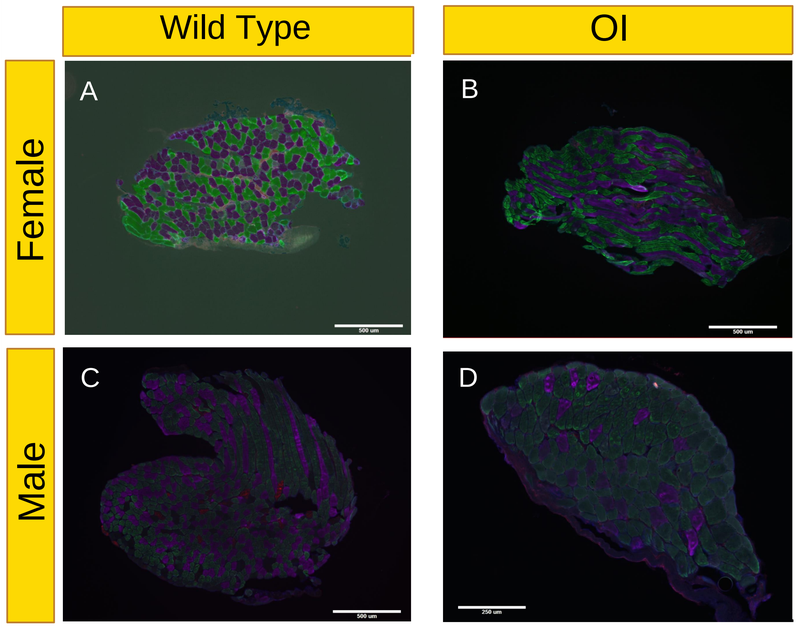

Figure 1: Representative images of immunohistological analyses of soleus muscles from wild type and OI mice. A is a wild type female, B is an OI female, C is a wild type male, and D is an OI male. Type I fibers are stained magenta, type IIa are stained green, and type IIb fibers are stained red. A, B, C are under 5X magnification and D is under 10X in order to show detail.

Myofiber Composition

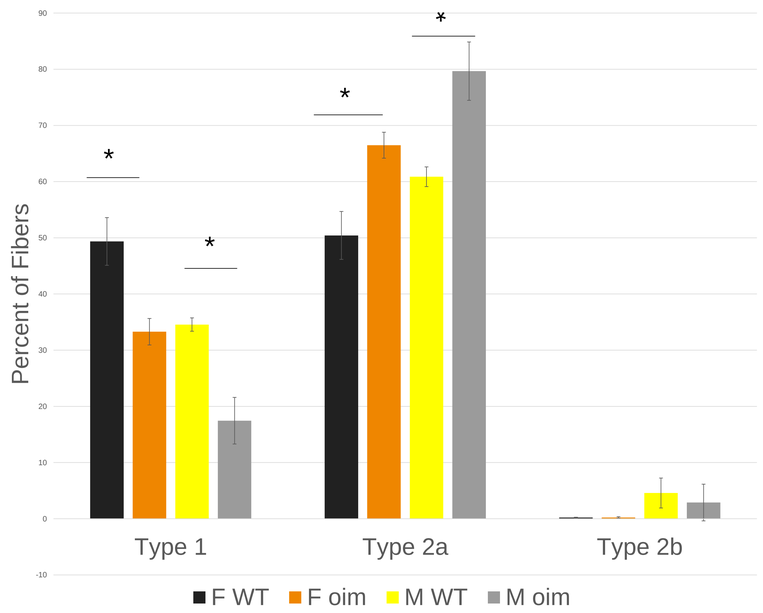

Figure 2: Myofiber types I, IIa and IIb composition in wild type and *oim/oim* soleus muscles. Female wild type (F WT, *black bar*, n=4), female *oim/oim* (F oim, *orange bar*, n=5), male wild type (M WT, *yellow bar*, n=4), and male *oim/oim* mice (M oim, *gray bar*, n=4). Columns indicate percentage of type I myofibers (*left*), type IIa myofibers (*center*), and type IIb myofibers (*right*).* indicates $p<0.05$.

Results

- *oim/oim* mice have significantly less type I muscle fibers than wild type mice in females and in males
- *oim/oim* mice have significantly more type IIa fibers than wild type mice in both females and males

Discussion

As muscle strength can build stronger bones through mechanotransduction, it is important to elucidate why patients with OI have inherently weaker muscles. In this study, we found that muscle fiber-type switching occurred in mice with an *oim/oim* genotype, modeling a severe, type III OI phenotype. The ratio of type I fibers to type IIa fibers was lower in *oim/oim* mice compared to wild type mice. More experiments need to be conducted in order to determine if muscle fiber-type switching occurs in less severe models of OI and if switching occurs in muscles other than the soleus.

References

1) Marini, Joan C., et al. “Osteogenesis Imperfecta.” Nature Reviews Disease Primers, vol. 3, no. 1, 2017, p. 17052, doi:10.1038/nrdp.2017.52

2)Gentry, Bettina A., et al. “Skeletal Muscle Weakness in Osteogeneis Imperfecta Mice.” Matrix Biology, vol. 29, no. 7, 2010, pp. 638–44, doi: https://doi.org/10.1016/j.matbio.2010.06.006.

3) Frost, H. M. “Perspectives: A Proposed General Model of the ‘Mechanostat’ (Suggestions from a New Skeletal-Biologic Paradigm).” The Anatomical Record, vol. 244, no. 2, John Wiley & Sons, Ltd, Feb. 1996, pp. 139–47, doi:10.1002/(SICI)1097-0185(199602)244:2<139::AID-AR1>3.0.CO;2-X.

4) Gremminger, V.L., Jeong, Y., Cunningham, R.P., Meers, G.M., Rector, R.S. and Phillips, C.L. (2019), Compromised Exercise Capacity and Mitochondrial Dysfunction in the Osteogenesis Imperfecta Murine (oim) Mouse Model. J Bone Miner Res, 34: 1646-1659. doi:10.1002/jbmr.3732

5) Schiaffino, Stefano, and Carlo Reggiani. “Fiber Types in Mammalian Skeletal Muscles.” Physiological Reviews, vol. 91, no. 4, American Physiological Society, Oct. 2011, pp. 1447–531, doi:10.1152/physrev.00031.2010

Acknowledgements

Leda J. Sears Trust Foundation, NIH/NIAMS R01 AR055907, March of Dimes, University of Missouri IMSD-EXPRESS R25GM056901, and the University of Missouri Cytology Core. Emily Harrelson was funded by a CAFNR On Campus Research Internship.