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Skeletal muscle weakness in osteogeneis imperfecta mice ☆

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ABSTRACT

Exercise intolerance, muscle fatigue and weakness are often-reported, little-investigated concerns of patients with osteogenesis imperfecta (OI). OI is a heritable connective tissue disorder hallmarked by bone fragility resulting primarily from dominant mutations in the proα1(I) or proα2(I) collagen genes and the recently discovered recessive mutations in post-translational modifying proteins of type I collagen. In this study we examined the soleus (S), plantaris (P), gastrocnemius (G), tibialis anterior (TA) and quadriceps (Q) muscles of mice expressing mild (+/*oim*) and moderately severe (*oim*/*oim*) OI for evidence of inherent muscle pathology. In particular, muscle weight, fiber cross-sectional area (CSA), fiber type, fiber histomorphology, fibrillar collagen content, absolute, relative and specific peak tetanic force (P_o, P_o/mg and P_o/CSA respectively) of individual muscles were evaluated. *Oim*/*oim* mouse muscles were generally smaller, contained less fibrillar collagen, had decreased P_o and an inability to sustain P_o for the 300-ms testing duration for specific muscles; +/*oim* mice had a similar but milder skeletal muscle phenotype. +/*oim* mice had mild weakness of specific muscles but were less affected than their *oim*/*oim* counterparts which demonstrated readily apparent skeletal muscle pathology. Therefore muscle weakness in *oim* mice reflects inherent skeletal muscle pathology.

1. Introduction

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder characterized by small stature, reduced bone mineral density, and frequent fractures (Byers, 1993). Greater than 85% of patients with OI fall into four (types I–IV OI) of nine potential subtypes, due to predominantly dominant mutations in either of the type I collagen genes, COL1A1 and COL1A2 (Basel and Steiner, 2009; Marini et al., 2007b). Though the gene defects responsible for types V and VI remain unknown, recent discoveries have attributed the rare recessive types (VII–IX) of OI to mutations in collagen post-translational modifying enzymes and proteins including prolyl-3-hydroxylase-1 (LEPRE1), cartilage-associated protein (CRTAP) and peptidyl-prolyl isomerase B (PPIB) genes, respectively (Baldridge et al., 2008; Barnes et al., 2006; Barnes et al., 2010; Cabral et al., 2007; Marini et al., 2007a; Morello et al., 2006; Van Dijk et al., 2009; Willaert et al., 2009). Of the four classical types, type I OI is the mildest clinically and is characterized by blue sclerae, premature deafness, and mild to moderate bone fragility. Type II is perinatal lethal and type III OI (the most severe viable form) is characterized by short stature, deformity of the long bones and spine due to fractures, as well as premature hearing loss (Gajko-Galicka, 2002). Type IV OI has a moderate variable phenotype between types I and III.

Fatigue and muscle weakness are also associated with OI (Engelbert et al., 1997; Takken et al., 2004), and can be the presenting symptom in patients with the disease (Boot et al., 2006). A case study of a patient with OI performed by Boot et al. documented increased acid phosphatase and swollen skeletal muscle mitochondria. However, muscle morphology, nerve conduction studies and electromyography were normal (Boot et al., 2006). This led Boot to conclude that "the etiology of decreased muscle force in patients with OI is unclear but may be due to an intrinsic muscle defect" (Boot et al., 2006). Takken at al. studied cardiopulmonary fitness and muscle strength in children with OI type I. They found that while no pulmonary or cardiac abnormalities at rest could be found, type I patients

Abbreviations: ATP, adensosine triphoshate; CSA, cross-sectional area; CRTAP, cartilage-associated protein; G, gastrocnemius; LEPRE1, prolyl-3-hydroxylase-1; LSD, least significant difference; OI, osteogenesis imperfecta; *oim*, osteogenesis imperfecta murine; P, plantaris; P_o, absolute whole muscle peak tetanic force; P_o/mg, relative muscle tetanic force; P_o/CSA, specific muscle tetanic force; PPIB, peptidyl-prolyl isomerase B; S, soleus; SE, standard error; SOCE, store-operated Ca^{+2}; TA, tibialis anterior; Q, quadriceps; VO_{2peak}, peak oxygen consumption; wt, wildtype.

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had decreased exercise tolerance and muscle strength compared to reference values for healthy pediatric peers (Takken et al., 2004). These findings were attributed to a combination of proximal muscle weakness and joint hypermobility, but it remained unclear whether the reduced peak oxygen consumption (VO_{2peak}) and muscle force were a consequence of inactive lifestyle or a specific consequence of the impaired type I collagen synthesis.

The purpose of this study is to determine if an inherent skeletal muscle defect exists in the osteogenesis imperfecta murine (*oim*) mouse model. The *oim* mouse is the most widely used model of osteogenesis imperfecta and was first described in 1993. *Oim*/*oim* are homozygous for a spontaneous nucleotide deletion which causes a frameshift in the COL1A2 gene resulting in the absence of functional α2(I) chains of type I collagen. *Oim*/*oim* mice produce exclusively homotrimeric type I collagen [$\alpha1(I)_3$] (Chipman et al., 1993) instead of heterotrimeric type I collagen, $\alpha1(I)_2\alpha2(I)$. The *oim*/*oim* mouse phenotype most closely correlates with human OI type III. Heterozygous (+/*oim*) mice have a milder phenotype similar to human OI type I (Camacho et al., 1999; McBride et al., 1998; Phillips et al., 2000; Saban et al., 1996),with bone biomechanical integrity intermediate to wildtype (wt) and *oim*/*oim* mice (Saban et al., 1996).

Though the impact of type I collagen mutations on bone strength and integrity is clearly evident (Byers, 2001; Marini et al., 2007b), whether type I collagen mutations impact skeletal muscle structure and function is unknown. In this study we investigated skeletal muscle characteristics of four month old age-matched male and female wildtype (wt), +/*oim* and *oim*/*oim* mice. We examined skeletal muscles with different fiber types and functional demands [soleus (S), plantaris (P), gastrocnemius (G), tibialis anterior (TA) and quadriceps (Q) muscles] and determined muscle weights, fibrillar collagen content, fiber cross-sectional area (CSA), fiber-type distribution, fiber histomorphology, and peak tetanic force (P_o) of individual muscles.

2. Materials and methods

2.1. Experimental model

Mature male and female wt, +/*oim* and *oim*/*oim* mice maintained on a C57BL/6J background (Carleton et al., 2008) were evaluated. However, as there was no apparent sex predilection for the muscle phenotype described and similar findings in males and females, only data from male mice are presented. Mice were 4 months of age at time of study to avoid the characterization of animals in the rapid growth phase of development. In addition, 4 months of age is when peak bone mass in mice is achieved and extensive studies have been performed characterizing the bone in *oim* mice at this age (Carleton et al., 2008; Phillips et al., 2000). The protocols used for this study comply with the guidelines of the American Physiological Society. All experimental manipulations were performed under an approved University of Missouri Animal Care and Use Protocol.

2.2. Contractile properties

The soleus (S), plantaris (P), gastrocnemius (G), tibialis anterior (TA), and quadriceps (Q) muscles were chosen based on their differing fiber-type compositions, architecture and contribution to movement. The S, P, G, TA and Q muscles are uni- or multi-pennate; span one or more joints and function as anti-gravity, postural or locomotor muscles. The S, P and G muscles serve as plantar flexors and the TA is a dorsal flexor in mice (Hesselink et al., 2002). The Q extends the knee as well as flexes the hip (Lieber, 2002).

To determine contractile properties of the S, P, G, and TA muscles in wt, +/*oim* and *oim*/*oim* mice, the mice were anesthetized with pentobarbital sodium (0.15 ml pentobarbital with 0.85 ml saline) with 0.15 ml as the first injection. Anesthesia was maintained with 0.05 ml injections given as needed. Each mouse was placed side lying on a water-jacketed heating pad which maintained body temperature at 37 °C. The left S, P, G, and TA muscle were surgically exposed only at their distal insertions. The distal tendon of each muscle was attached in turn to the Grass force transducer with 4.0 silk. The sciatic nerve was isolated and placed on a bipolar stimulating electrode. The exposed tendon of each muscle was continually bathed in saline solution and the nerve was bathed continuously with 37 °C mineral oil.

For contractile testing the left hind limb and mouse torso were rigidly immobilized, and muscles were attached in the order of S → P → G → TA to a force transducer by the distal tendon and adjusted in length so that passive tension was zero grams. A twitch was obtained at that position with the parameters: 0.5 ms, 0.3 Hz, at 6 V, and subsequently the micromanipulator was used to progressively lengthen each muscle to the point where peak twitch was attained (L_o). At optimal length, a peak tetanic contraction (P_o) was elicited by pulses delivered at 150 Hz, 300-ms duration, and an intensity of 6 V for each muscle (Brown et al., 2009). Preliminary studies revealed 6 V to be supramaximal; the 300-ms duration was greater than what was required to achieve P_o. Force curves generated at 15, 50, 75, 100 and 125 Hz revealed that all muscles were maximally recruited by the time 100 Hz was reached. All data were collected using Power Lab®. The duration of contractile function testing was approximately 15 min.

In pilot studies, nonsystematic testing of muscles was done as well as testing in the order TA → G → P → S and no differences in tension were observed, regardless of stimulation order. Repeat testing of S and P during preliminary studies and subsequently during actual stimulation indicated the protocol did not result in reduced force production.

2.3. Tissue harvest

After contractile properties were obtained, left (stimulated) S, P, G, TA, and Q muscles were removed, cleaned of extraneous tissue, blotted, and weighed. These muscles were then placed in 4% paraformaldehyde solution for 24 h followed by transfer to 70% ethanol for future staining with hematoxylin and eosin (H&E) for morphologic evaluation. Right-sided muscles, those that were not electrically stimulated, were placed at their *in situ* length, embedded in OCT tissue-freezing medium, frozen slowly in chilled 2-methylbuterol, and then placed in liquid nitrogen and stored at −80 °C until analysis for myofibrillar ATPase activity.

2.4. Histochemistry, cross-sectional myofiber area measurements, and fibrillar collagen content determination

Left-sided paraformaldehyde and ethanol prepared muscles from wt, +/*oim*, and *oim*/*oim* mice were transversely sectioned at the middle of the muscle belly and then sectioned at 5 μm and separate sections were stained with H&E to reveal evidence of potential muscle damage or inflammation or with picrosirius red stain to visualize and quantify fibrillar collagen content. From H&E stained sections fiber areas were obtained. Digital images were taken from the cross-section at 10x magnification to obtain an average of 300 fibers to evaluate muscle fiber morphology and for fiber area measures. Myofiber cross-sectional areas were used to measure evidence of atrophy or hypertrophy of the individual muscle fibers, as well as in the calculation of specific P_o [peak tetanic force (P_o)/CSA (μm^2)]. Six sections of 50 contiguous myofibers were circled for each muscle evaluated to obtain an average of 300 fibers for fiber area measures. Area determinations were done using a calibrated pen by circling each fiber. Image J software (NIH) was used to derive area data which were subsequently transferred into an Excel spreadsheet.

Digital images of muscle fibers (10× magnification) stained with picrosirius red stain were captured using an Olympus D11 digital camera (Olympus America Inc., NY) attached to a Zeiss (Carl Zeiss Inc., NY) microscope. Images were opened in Adobe Photoshop and the tiff files were used for morphological studies of fibrillar collagen content using Fovea Pro v.3 from Reindeer Graphics (P. O. Box 2281 Asheville, NC 28802). Fibrillar collagen content was determined using BiLevel