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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.

Keywords

muscle weight; peak tetanic force; skeletal muscle; type I collagen

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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 et al., 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 mutations in collagen posttranslational 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 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 (0.15ml pentobarbital with 0.85ml saline) with 0.15ml as first injection, and anesthesia was maintained with 0.05ml injection 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: 0.5ms, 0.3Hz, at 6V, 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 150Hz, 300-ms duration, and an intensity of 6V for each type muscle (Brown et al., 2009). Preliminary studies revealed 6V 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 minutes.

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 hours 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 (10X 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 Thresholding with the threshold value of 50 to ensure optimal tissue:fibrillar collagen contrast. Total area of the muscle tissue was determined by subtracting out surrounding empty space in the visible field. Artifacts (marking ink, tissue folds, birefringent muscle fibers, large, brightly staining vessels, and epimysium when visible) were blackened out and fibrillar collagen content was calculated (mm^2) corresponding to picrosirius red staining. Fovea Pro.software was used to derive area data which were subsequently transferred into an Excel spreadsheet. Fibrillar collagen area was divided by total muscle tissue area to determine the percent collagen:muscle tissue area ratio for each muscle.

2.5 Myofibrillar ATPase activity

Right-sided S, P, G, TA and Q muscles, which were harvested immediately after contractile studies and stored at −80°C, were thawed to −20°C in a microtome, oriented vertically, and sectioned at 10 μm. Sections were stained using traditional acid-stable myofibrillar ATPase stain (4.2 $_p$H acid preincubation) to reveal muscle fiber type (Carson, 1997).

2.7 Statistical analysis

All statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC). Data from the three groups (males and females) were analyzed as a 3×1 factorial (3 genotypes, 1 sex). When heterogeneous variations made it necessary, a log transformation was used to stabilize heterogeneous variation. If this log transformation failed to stabilize the variation, a ranking procedure was used (Conover et al., 1982). Mean differences were determined using Fisher's Least Significant Difference (LSD). Means and standard errors presented are untransformed values, although P values are for the transformed or ranked data. All results are presented as mean ± standard error (SE). Differences were considered to be statistically significant at a p

value ≤0.05. Data obtained for male and female mice were nearly identical, and so only findings from males are included in the results.

3. Results

3.1 Muscle Weight

Consistent with previous studies (Carleton et al., 2008; Chipman et al., 1993; McBride et al., 1997), o*im*/*oim* mouse body weights were significantly less ($p < 0.05$) than the body weights of wt and +/*oim* littermates (Table 1). Whole muscle wet weights for the S, P, G, TA and Q muscles in *oim*/*oim* mice were 77%, 70%, 70%, 78% and 82% ($p < 0.05$) of their wt counterpart muscle weights, respectively. Whole muscle weights of +/*oim* S, P, G, TA and Q muscles were 97%, 94%, 98%, 95% and 99% of wt whole S, P, G, TA and Q weights (not significant). Whole muscle weights for the S, P, G, TA and Q muscles in *oim*/*oim* mice were 80%, 76%, 72%, 72%, 82% and 83% ($p < 0.05$) of +/*oim* mice, respectively. In addition, relative muscle wet weights (expressed as a percent body weight) of *oim*/*oim* P, G and TA muscles were 82%, 82% and 92% of wt, respectively. Relative muscle weights of +/*oim* S, P, G, TA and Q muscles were 99%, 97%, 100%, 97% and 101% of relative weights of wt S, P, G, TA and Q (not significant). Relative muscle weights of *oim*/*oim* S, P, G, and TA muscles were 90%, 85%, 82% and 94% ($p < 0.05$) compared to +/*oim* relative muscle weights (Table 1).

3.2 Histomorphometry

Histology cross-sections of the S, P, G, TA and Q muscles stained with hematoxylin and eosin (H&E) showed no evidence of necrosis, regeneration, degeneration, fibrosis or infiltration by inflammatory cells (Fig. 1).

3.3 Muscle fibrillar collagen content

Fibrillar collagen content of the *oim*/*oim* S muscle was less ($p < 0.05$) compared to wt. Fibrillar collagen content of the Q muscle in *oim*/*oim* and +/*oim* mice was less ($p < 0.05$) compared to wt. Fibrillar collagen content was not different in the G or TA muscles between the genotypes (Fig. 2). Fibrillar collagen as a whole represented less than 3.5% of the tissue area (mm^2).

3.4 Myofibrillar ATPase

Fiber-type analysis of the S, P, G, TA and Q muscles of wt, +/*oim* and *oim*/*oim* mice by histochemical staining for ATPase activity (acid preincubation) revealed no significant differences in the absolute number of slow fibers in +/*oim* or *oim*/*oim* mice as compared to wt muscles (Fig. 3A,B).

3.5 Myofiber Cross sectional Area

Evaluation of cross-sectional area (CSA) of the muscle fibers of the S, P, G, TA and Q muscles indicate that *oim* mice do not have smaller muscle fiber CSAs than wt mice. Interestingly, *oim*/*oim* mice did exhibit larger ($p < 0.05$) CSA of their Q myofibers compared to their +/*oim* counterparts (Fig. 4).

3.6 Contractile Force Generating Capacity

Absolute whole muscle contractile generating capacity [peak tetanic force (P_o)] was less in *oim*/*oim* S ($p \leq 0.055$), G and TA ($p < 0.05$) muscles compared to wt and +/*oim* muscles. P_o for P was significantly impaired in +/*oim* and *oim*/*oim* compared to wt (Fig. 5A). Relative muscle contractile generating capacity [peak tetanic force (P_o) / muscle weight in milligrams (mg)] was impaired ($p < 0.05$) in the TA muscle of *oim*/*oim* mice relative to their wt and +/*oim* counterparts (Fig. 5B). Specific P_o [peak tetanic force (P_o) / CSA (μm^2)] was decreased ($p < 0.05$) in *oim*/*oim* G and TA muscles compared to wt and +/*oim* (Fig. 5C). P P_o/CSA was

reduced ($p < 0.05$) in +/*oim* and *oim*/*oim* relative to wt mice (Fig. 5C). In addition to determining the contractile force generating capacity, we observed a severe decay of tetanic force in the S, G and TA muscles of *oim*/*oim* mice and a mild decay of tetanic force in the G and TA muscles of +/*oim* relative to wt mice (Fig. 6). The *oim* muscles could not sustain P_o for the 300 ms duration. A representative example of this decay and inability to sustain P_o during 300 ms of stimulation can be seen with the force curves of the TA of +/*oim* and *oim*/*oim* mice (Fig. 6). The equivalent shape and duration of the toe region in contractile force recordings of the S, P, G and TA between wt, +/*oim* and *oim*/*oim* mice, and passive tensions, suggested that there was no difference between the genotypes in the ability of the tendon to maintain tension (data not shown).

4. Discussion

We found that *oim*/*oim* mice had and weaker muscles compared to their wt and +/*oim* counterparts. Since *oim*/*oim* mice are significantly smaller than their wt littermates (Carleton et al., 2008; Chipman et al., 1993; Phillips et al., 2000), we anticipated that muscle weight would also be decreased. Though whole muscle wet weights of *oim*/*oim* mice were 75% and 80% of wt and +/*oim* mice, respectively, the muscles remained smaller even when normalized to body weight; *oim*/*oim* relative muscle weights were approximately 88% and 90% of wt and +/*oim* muscles, respectively. This percent loss of whole and relative muscle weight is of potential clinical significance and suggests that reported muscle weakness by OI patients with moderate to severe forms of the disease may be due, in part, to decreased muscle size. Currently the biological significance of these findings is unknown, and the percent loss of muscle that correlates to a subsequent loss of function is not well characterized in mice or humans. Female mice exhibited the same trends as males and had similar decreases in wet muscle weights and contractile capacity. There appears no predilection in clinical presentation of the disease for either males or females.

There were no differences in the distribution of muscle fiber types in the S, P, G, TA and Q muscles between wt, +/*oim* or *oim*/*oim* genotypes, suggesting that neither inherent differences in fiber type nor fiber type switching can account for the observed increased CSA of the Q or decreased contractile function of the S, P, G, or TA muscles in +/*oim* or *oim*/*oim* mice. Mice have primarily a type II fiber makeup (Burkholder et al., 1994) even for muscles that are considered “slow twitch” such as the soleus muscle (Glaser et al., 2009). A limitation of the ATPase methodology for muscle fiber typing is that only type I and type II fibers are clearly distinguishable, and thus, subtle differences in type II fiber subtype composition can not be ruled out (Burkholder et al., 1994; Lieber, 2002).

Though relative muscle weight was decreased in most of the *oim*/*oim* muscles compared to wt and +/*oim* mice, CSA did not appear negatively affected in *oim*/*oim* mice. In particular, while whole muscle weights of the Q muscle in *oim*/*oim* male mice were decreased relative to wt and +/*oim* littermates, the relative Q muscle weight was not reduced and there was even an increase in average Q myofiber CSA.

Fiber area affects fiber force and fiber type distribution affects muscle speed and endurance (Lieber, 2002). Skeletal muscle contractile properties depend on many factors including muscle fiber size, fiber properties (i.e. cross-bridge formation, Ca^{+2} influx into the sarcoplasmic reticulum), and the arrangement and number of fibers within the muscle (i.e., muscle architecture) (Burkholder et al., 1994). Carleton et al. have shown that *oim*/*oim* femur lengths are 3-4% shorter than wt femurs (Carleton et al., 2008), whereas tibial lengths between wt, +/*oim* and *oim*/*oim* were not different (data not shown). Perhaps the *oim*/*oim* Q fiber CSA reflects altered bone geometry and / or altered muscle attachment sites and architecture which are not present in the tibia associated muscles, the S, P, G and TA.

Decreased muscle strength may also result from a decrease in skeletal muscle capillary density. Takken et al. found that OI children had significantly lower peak oxygen consumption (VO_{2peak}) compared with healthy subjects, though there was no evidence of circulatory abnormalities (Takken et al., 2004). It has been noted that subjects which are more sedentary compared to those with active lifestyles have fewer capillaries in their muscles per CSA (Takken et al., 2004). Sedentary subjects also have a reduction of oxidative enzyme which can result in reduced skeletal muscle capacity for oxygen extraction from the blood, leading to reduced VO_{2peak} (Takken et al., 2004). Activity levels and capillary density in *oim* mice remain to be evaluated.

The P muscle of +/*oim* mice was significantly weaker compared to wt. In addition, +/*oim* mice displayed a mild decay of tetanic tension during the stimulation period. This indicates that +/*oim* mice (representing individuals with clinically mild OI type I) may have mild skeletal muscle weakness, consistent with findings by Takken et al. (Takken et al., 2004). Although no obvious disruptions in the muscle cell morphology were noted, mitochondria and enzymatic parameters were not examined. In addition to the reduced P_o in S, G and TA muscles of *oim*/*oim* compared to wt and +/*oim* mice, as well as the decreased P_o/CSA in *oim*/*oim* P, G and TA muscles compared to wt mice, we observed a decay of tension during tetanic stimulation in the TA, S and G muscles in *oim*/*oim* mice, which was less apparent in the P muscle of *oim*/*oim*. We also observed a mild decay of tension in both the TA and G muscles of +/*oim* which lead us to believe that the skeletal muscle deficits observed have physiologic causes (Fig. 6). These phenomena may be due to alterations in the regulation of intracellular Ca^{+2} handling and thus muscle contractility. There may also be reduced or altered functional status of store-operated Ca^{+2} (SOCE) resulting in defective Ca^{+2} homeostasis in *oim*/*oim* muscle cells. It would be valuable to perform single muscle studies in order to address this phenomenon.

We also observed reduced levels of fibrillar collagen in *oim*/*oim* skeletal muscle compared to wt and +/*oim*, which is consistent with the markedly lower myocardial collagen content and reduced collagen fiber diameter found in the *oim*/*oim* heart, contributing to decreased ventricular function (Weis et al., 2000).

Tendons of *oim*/*oim* mice are also known to have reduced type I collagen levels and to be biomechanically compromised (Misof et al., 1997; Sims et al., 2003). Tail tendon of *oim*/*oim* mice contains approximately 60% less collagen and possesses 40% lower tensile strength than wt controls, and *oim*/*oim* tendon fibers are thinner than age-matched and weight-matched controls (McBride et al., 1997).

Force transduction measurements in our experiments were performed by attaching silk thread to the tendons in the *oim* mice, which may have resulted in possible discrepancies in the amount of force measured due to alterations in type I collagen strength and content. However, examination of the contractile force recordings and passive tensions of wt, +/*oim* and *oim*/*oim* S, P, G and TA muscles suggest that there is no difference between the genotypes in the ability of the tendon to maintain tension.

Although the *oim* mouse is the most widely used mouse model of osteogenesis imperfecta, the *oim* gene defect, which causes the absence of functional α2(I) chains and it's recessive mode of inheritance are rare causes of OI in the human population (Nicholls et al., 1984; Pihlajaniemi et al., 1984).

We demonstrate that *oim*/*oim* mice, which are phenotypically similar to the clinical presentation of type III OI in humans, have impairment of certain hindlimb muscles. Although all muscles evaluated appear morphologically normal, whole muscle wet weights and fibrillar collagen content of the S and Q muscles are significantly reduced compared to their wt and +/*oim* counterparts. P, G and TA muscles of *oim*/*oim* mice are reduced in weight compared to

wt and +/*oim* mice to a greater extent than what would be expected due to their overall decreased body size. P_o of all muscles evaluated is compromised in *oim*/*oim* mice. Muscle fiber type distribution is similar for all muscles evaluated between genotypes. It appears also that +/*oim* mice (whose phenotype is similar to the mild form of OI type I in humans) have mild weakness of specific muscles and are less affected than their *oim*/*oim* counterparts, which have readily apparent skeletal muscle pathology. Our findings suggest that the skeletal muscle weakness in *oim* mice may reflect the decreased muscle strength observed in patients with OI (Boot et al., 2006; Takken et al., 2004). Suskauer et al. looked at physical performance in children with OI and found that they were significantly less active than their nondisabled peers (Suskauer et al., 2003). In children the cycle of fractures, pain and casting likely leads to minimized activity. Although it is also possible that reduced physical activity compounds the inherent skeletal muscle pathology in *oim* mice, the fiber CSAs were not different for *oim*/*oim* mice compared to controls, which undermines the potential contribution of reduced physical activity as an explanation for our findings. Future studies are needed to investigate muscle weakness at the single fiber and ultrastructural levels. Additionally, further investigations are needed of skeletal muscle in other mouse models of OI such as *Brtl* (Forlino et al., 1999) and *G610C* (Daley et al., 2009) as well as the CRTAP deficient (Morello et al., 2006) and Cyclophilin-B deficient mice (Choi et al., 2009) to clarify the role of OI causing mutations on skeletal muscle weakness and function.

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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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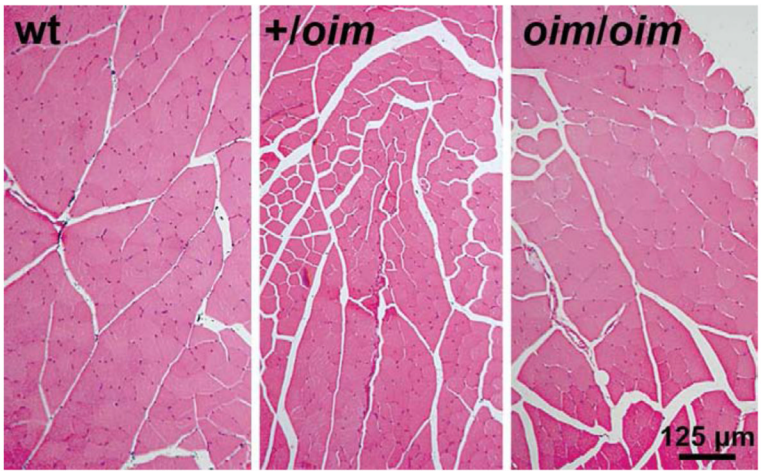

Figure 1.
Hematoxylin and eosin (H&E) stained cross sections of the tibialis anterior (TA) muscle from 4 month old wt *(left)*, +/*oim(middle)* and *oim*/*oim(right)* mice showed no evidence of necrosis, regeneration, degeneration, or infiltration by inflammatory cells, 10X magnification.

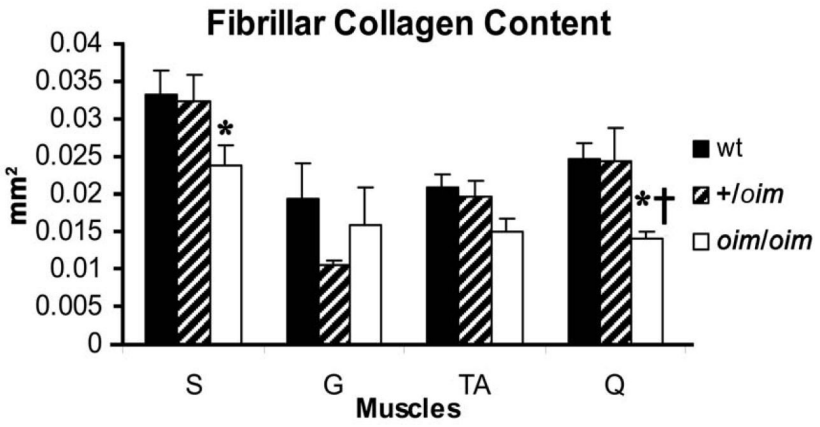

Figure 2.

Fibrillar collagen content as determined by picrosirius red staining of the S, G, TA and Q muscles of wt (n=6; *solid*), +/*oim* (n=6; *dashed*) and *oim*/*oim* (n=6; *open*) mice. *Oim*/*oim* S had less ($p < 0.05$) collagen compared to wt S. Q had significantly less ($p < 0.05$) collagen compared to wt and +/*oim* Q.

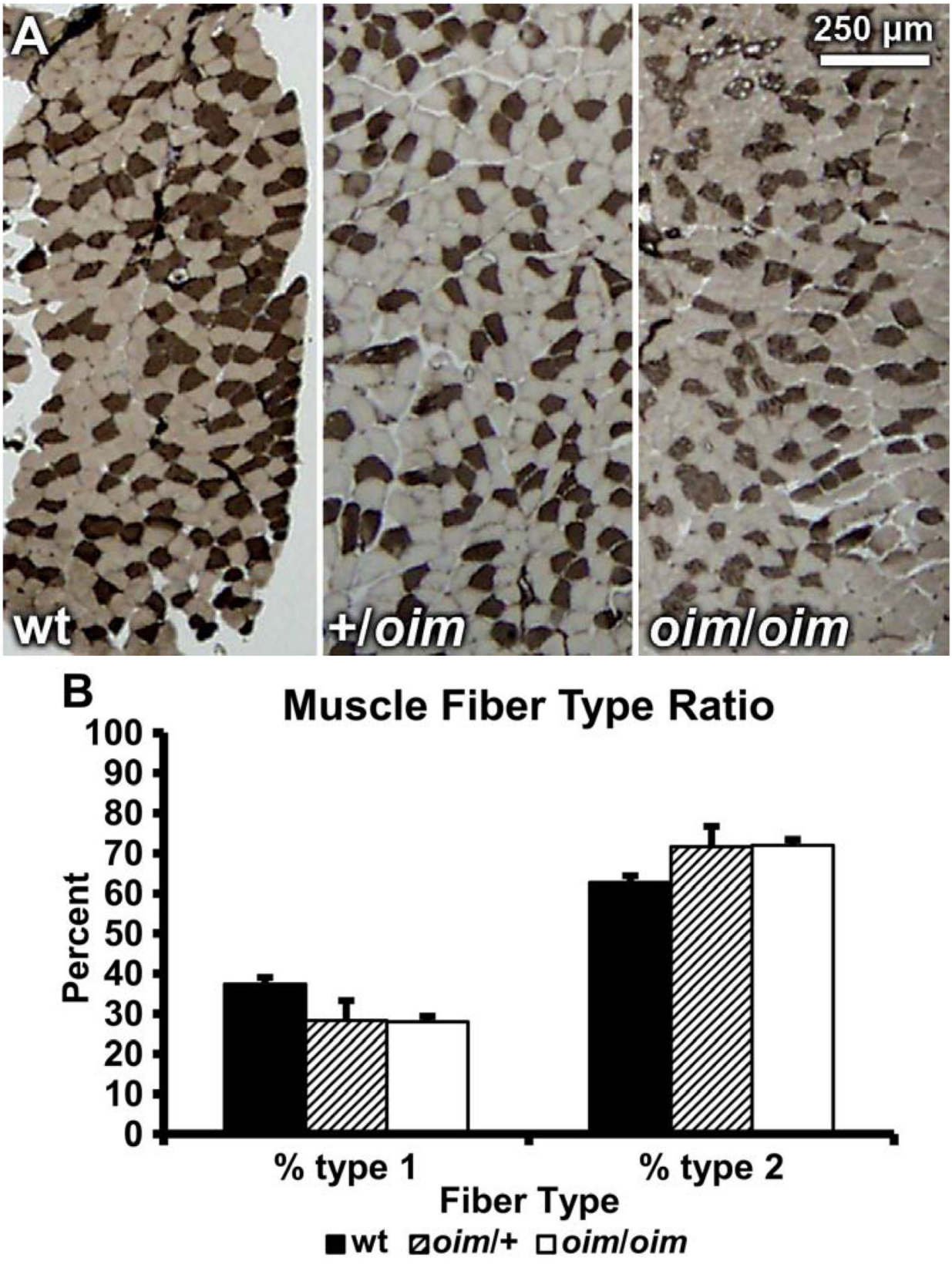

Figure 3.

(A) Myofibrillar ATPase stained (acid preincubation) +/*oim* (n=3; *center*) and *oim*/*oim* (n=4; *right*) soleus (S) muscle from 4 month old mice did not show differences in distribution of type I or type II fibers compared to wt (n=3; *left*) S muscle, 2.5X magnification. The same was true for plantaris (P), gastrocnemius (G), tibialis anterior (TA) and quadriceps (Q) muscles (results not shown). **(B)** Distribution of type I and type 2 fibers of the S muscle expressed as a percent of total muscle fiber number (mean ± SE).

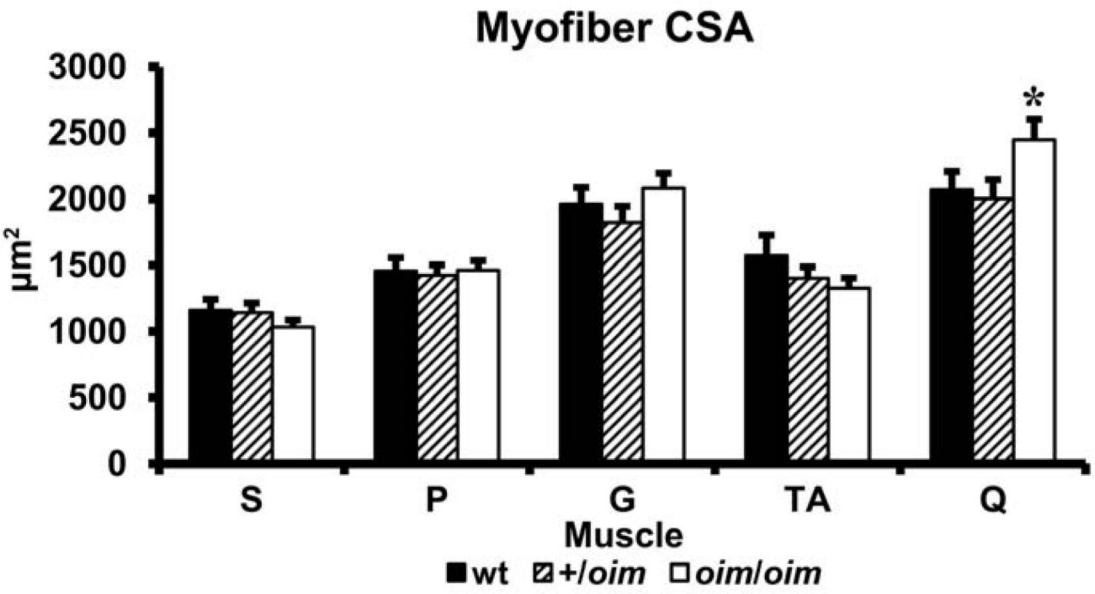

Figure4.

Cross-sectional area (CSA (μm^2)) of soleus (S), plantaris (P), gastrocnemius (G), tibialis anterior (TA) and quadriceps (Q) muscles of wt (n=12; *solid*), +/*oim* (n=14; *diagonal*), and *oim*/*oim* (n=13; *open*) mice (mean ± SE). Q CSA is significantly larger in *oim*/*oim* mice compared to their +/*oim* counterparts. * $p < 0.05$ compared to +/*oim*.

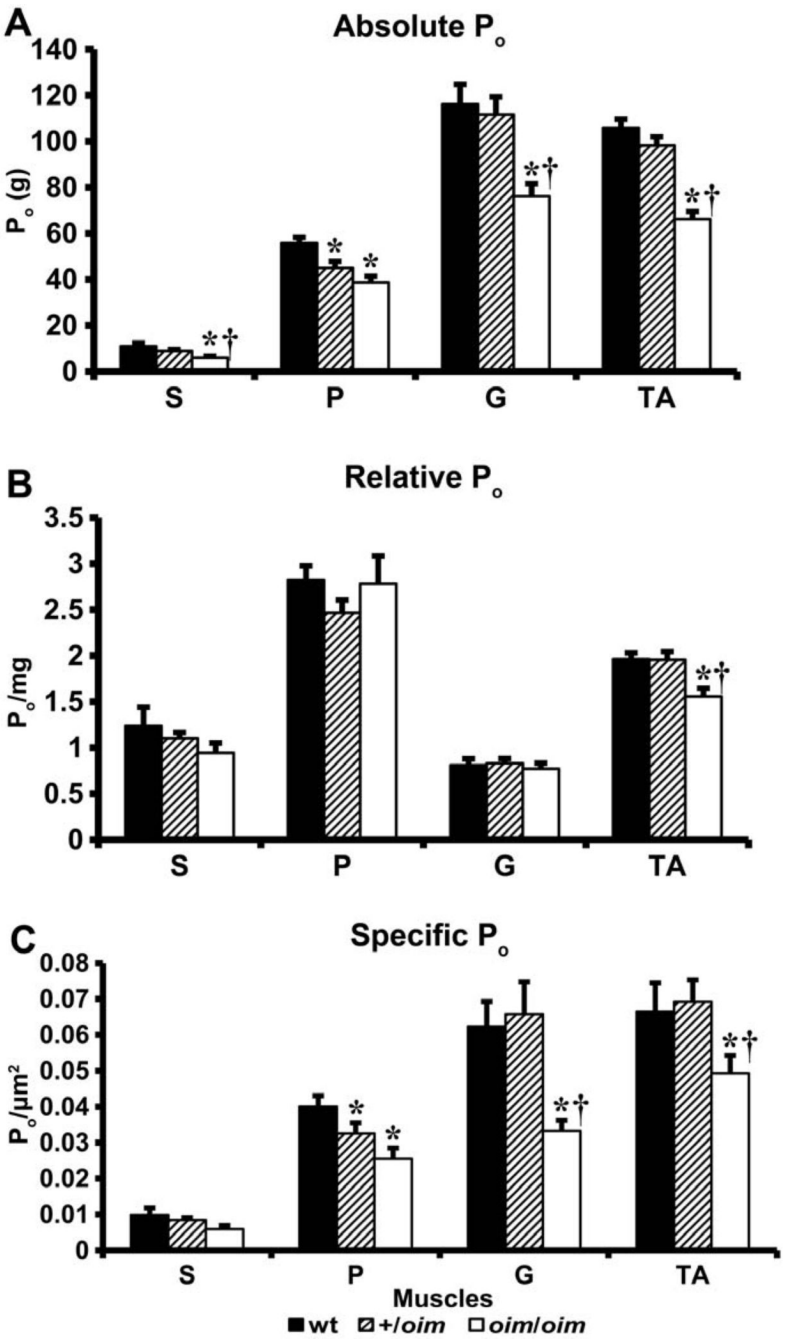

Figure 5.

(**A**) Absolute tetanic force (P_o) for soleus (S) ($p < 0.055$), gastrocnemius (G) and tibialis anterior (TA) muscles is reduced in 4 month old *oim*/*oim* (n=11; *open*) compared to wt (n=12; *solid*) and +/*oim* (n=12; *diagonal*) mice. P_o for the plantaris (P) is reduced in +/*oim* and *oim*/*oim* mice compared to wt (mean ± SE). (**B**) Relative tetanic force (P_o / mg) for the TA muscle is significantly reduced in *oim*/*oim* mice compared to their +/*oim* and wt counterparts (mean ± SE). (**C**) Specific tetanic force (P_o / CSA) is significantly less in the P of +/*oim* and *oim*/*oim* mice compared to wt. Po/CSA of G and TA muscles is significantly less in *oim*/*oim* compared to wt and +/*oim* mice (mean ± SE). * $p < 0.05$ compared to wt, † $p < 0.05$ compared to +/*oim*

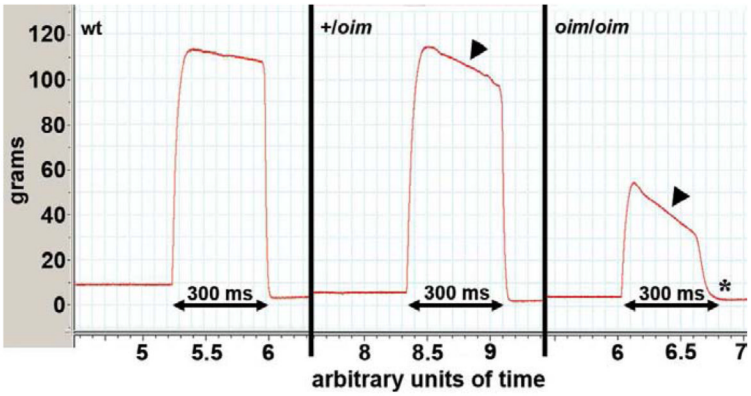

Figure 6.

Tetanic stimulation of the tibialis anterior (TA) muscle of 4 month old mice shows a precipitous decay in tetanic force in *oim*/*oim* (*right, arrowhead*) and mild decay in +/*oim* (*center, arrowhead*) compared to wt (*left*) mice (300 ms duration, 150 Hz). In addition, *oim*/*oim* mice were unable to sustain P_o contraction during the entire 300 ms stimulation period (*right, asterisk*).

Table 1

Body and muscle weight of four month old wt, *+/oim* and *oim/oim* mice.

	MOUSE GENOTYPE					
PARAMETERS	**wt (n=13)**	***+/oim* (n=17)**	***+/oim* % wt values**	***oim/oim* (n=13)**	***oim/oim* % wt values**	***oim/oim* % *+/oim* values**
Body weight (g)	29.53 ± 0.63	28.88 ± 0.56	98	25.30 ± 0.63*†	86	88
Muscle wet weight (mg)						
S	8.51 ± 0.31	8.26 ± 0.26	97	6.58 ± 0.31*†	77	80
P	20.16 ± 0.51	19.01 ± 0.44	94	14.17 ± 0.51*†	70	76
G	144.04 ± 3.87	140.91 ± 3.38	98	101.17 ± 3.87*†	70	72
TA	54.32 ± 1.17	51.77 ± 1.03	95	42.55 ± 1.17*†	78	82
Q	227.44 ± 6.68	225.03 ± 5.84	99	186.88 ± 6.68*†	82	83
Relative Wet Muscle Weight [Wet wt (mg)/ Body wt (g)]						
S	0.029 ± 0.001	0.029 ± 0.001	99	0.026 ± 0.001†	89	90
P	0.068 ± 0.001	0.066 ± 0.001	97	0.056 ± 0.001*†	82	85
G	0.489 ± 0.012	0.490 ± 0.010	100	0.400 ± 0.012*†	82	82
TA	0.184 ± 0.004	0.180 ± 0.003	97	0.169 ± 0.004*†	92	94
Q	0.771 ± 0.021	0.781 ± 0.019	101	0.741 ± 0.021	96	95

Values expressed as mean ± SE. G, gastrocnemius; oim, osteogenesis imperfecta murine; P, plantaris; S, soleus; TA, tibialis anterior; Q, quadriceps;

* $p < 0.05$ compared to wt,

† $p < 0.05$ compared to *+/oim*