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## Soluble activin receptor type IIB decoy receptor differentially impacts murine osteogenesis imperfecta muscle function

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### Abstract

**Introduction**—Osteogenesis imperfecta (OI) is characterized by skeletal fragility and muscle weakness. We investigated the effects of soluble activin type IIB receptor (sActRIIB-mFc) on muscle mass and function in two distinct mouse models of OI, osteogenesis imperfecta murine (*oim*) and *+G610C* mouse.

**Methods**—Wildtype (WT), *+G610C*, and *oim/oim* mice were treated from 2 to 4 months of age with Tris-Buffered Saline (vehicle) or sActRIIB-mFc and their hindlimb muscles evaluated for mass, morphology and contractile function.

**Results**—sActRIIB-mFc-treated WT, *+G610C* and *oim/oim* mice had increased hindlimb muscle weights and myofiber cross-sectional area compared to vehicle-treated counterparts. sActRIIB-mFc treated *oim/oim* mice also exhibited increased contractile function relative to vehicle treated counterparts.

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#### Disclosure of Conflicts of Interest

The remaining authors have no conflict of interests.

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#### Authorship/Credit

Youngjae Jeong participated in research design, data acquisition and analyses, and drafting and revising of the article, Salah A. Daghlas, Alp S. Kahveci, R. Scott Rector, Daniel Salamango, and Marybeth Brown participated in data acquisition and analyses, Bettina A. Gentry and R. Scott Rector participated in data analyses, R. Scott Pearsall participated in research design, and Charlotte L. Phillips participated in research design, data analyses, and drafting and revising of the article. All authors have approved the final article and agreed to be accountable for all aspects of the work.

**Discussion**—Blocking endogenous ActRIIB was effective at increasing muscle size in mouse models of OI, and increasing contractile function in *oim/oim* mice. ActRIIB inhibitors may provide a potential mutation specific therapeutic option for compromised muscle function in OI.

### Keywords

osteogenesis imperfecta murine; *+G610C*; muscle; activin receptor type IIB; myostatin; peak tetanic force

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### Introduction

Sarcopenia is one of the most common pathophysiological outcomes associated with aging, resulting in reduced muscle mass with substantial changes in muscle fiber type from oxidative to glycolytic.<sup>1</sup> Consequences of reduced muscle mass and strength often lead to increased fall risk and fractures, which are in part strongly influenced by cross-talk of the muscle-bone units. Frost's mechanostat theory suggests that bone is mechanosensitive and that muscle mass and contractile force elicit peak voluntary mechanical load on bone.<sup>2</sup> Indeed, many patients with sarcopenia are likely to develop osteoporosis and osteopenia as well.<sup>3–9</sup> Sarcopenia and osteoporosis are not only associated with aging, but are also present in many disease states, including cancer cachexia, muscular dystrophies, and osteogenesis imperfecta (OI).<sup>10–12</sup>

Osteogenesis imperfecta, also known as brittle bone disease, is a heritable connective tissue disorder characterized by compromised biomechanical integrity in type I collagen containing tissues, including bone, vasculature, skin, and tendon.<sup>13</sup> More than 85% of individuals with OI have mutations in their type I collagen genes, *COL1A1* or *COL1A2*, while the remaining 15% have mutations in the genes of proteins associated with type I collagen assembly, mineralization or osteoblast function.<sup>13</sup> The majority of the clinical and basic research in OI has been targeted towards improving bone directly. Although the correlation between muscle and bone strength is well established, muscle function and pathology in OI has only just begun to be investigated. The limited number of studies evaluating muscle function in type I and type III OI patients report reduced muscle strength.<sup>14–17</sup> Muscle function has also been evaluated in two different mouse models of OI, and demonstrated that moderately severe recessive osteogenesis imperfecta murine (*oim*) mice have an inherent muscle pathology with reduced skeletal muscle mass and contractile function,<sup>12</sup> while the milder dominant negative OI mouse model, *+G610C*, does not.<sup>18</sup> Due to the importance of the muscle-bone crosstalk to bone strength and quality, it is critical to understand muscle properties in OI; as improved muscle function is known to decrease fracture risk and may likely lead to improved bone strength and quality.

Myostatin, a well-known negative regulator of muscle growth, is a member of the TGF- $\beta$  superfamily and binds to activin receptor type IIB to activate multiple intracellular signaling pathways to regulate gene expression.<sup>19</sup> Myostatin knock-out mice exhibit muscle hypertrophy and hyperplasia.<sup>20</sup> Due to myostatin's impact on muscle, various therapeutic molecules have been developed to inhibit myostatin and have been evaluated in a diverse group of muscle-related disorders.<sup>21–26</sup> Among these molecules, the soluble activin receptor

type IIB-mFc has shown promising results in age-related sarcopenia, cancer cachexia, muscle dystrophy induced by hypoxic conditions, and muscular dystrophic disease.<sup>22–25,27,28</sup>

The presence of muscle weakness in OI not only negatively impacts activities of daily living, but it may also contribute to skeletal fragility and increased fracture risk. In the following study, we sought to investigate whether treatment with the soluble activin receptor type IIB decoy molecule (sActRIIB-mFc) would increase muscle mass and enhance contractile function in two molecularly distinct mouse models of OI, osteogenesis imperfecta murine (*oim*) and *+G610C*. Homozygous *oim* (*oim/oim*) mice have a functional null mutation in their *Col1a2* genes, and thus compensate by making homotrimeric type I collagen,  $\alpha 1(I)_3$ , instead of normal heterotrimeric type I collagen,  $\alpha 1(I)_2\alpha 2(I)$ , while the *+G610C* mice are heterozygous for a glycine to cysteine substitution at position 610 of the  $\alpha 2$  chain of type I collagen.<sup>29,30</sup> Genetically and phenotypically, the *+G610C* mouse models osteogenesis imperfecta in affected family members of a large Amish kindred.<sup>31</sup> The *oim/oim* and the *+G610C* mice model moderately severe human OI type III and milder to moderate OI type IV individuals, respectively. As previously reported, *oim/oim* mice exhibit a muscle pathology, with reduced muscle mass and absolute and specific peak tetanic forces,<sup>12</sup> while the *+G610C* mice do not.<sup>18</sup> In the following study, we investigated the impact of sActRIIB-mFc on muscle properties of *+G610C* and *oim/oim* mice to test the hypothesis that blocking the ligands that bind the endogenous activin receptor IIB by the sActRIIB-mFc decoy will increase hindlimb skeletal muscle weights and absolute muscle contractile strength regardless of the presence of muscle pathologies associated with mutations in type I collagen (glycine substitution vs. functional null *Col1a2*).

## Methods

### Animals

Both OI mouse models, *G610C* (*Col1a2<sup>tm1.1Mchr</sup>*) and *oim* (*Col1a2<sup>oim</sup>*), were bred and maintained on the C57BL/6J congenic background by repeatedly backcrossing into wildtype C57BL/6J mice (stock number 000664, Jackson Laboratory, Bar Harbor, ME, USA) to minimize potential modifier gene effects. The mice were genotyped as previously described<sup>32,30</sup> and had *ad libitum* access to water and food (Purina 5008 Formulab Diet; Purina Mills Inc., St Louis, MO). The animals were housed in an AAALAC accredited facility at the University of Missouri and the protocols used for this study performed under an approved University of Missouri Animal Care and Use Protocol.

### Drug Administration

Male and female WT, *+G610C*, and *oim/oim* mice were each randomly distributed into two groups and treated bi-weekly with either vehicle [tris-buffered saline (TBS)] as control or 10mg/kg sActRIIB-mFc in TBS (RAP-031, Acceleron Pharma. Inc, Cambridge, MA) by intraperitoneal injection beginning at 2 months of age and continuing to 4 months of age. The 8–16 week treatment interval encompasses pubertal growth through skeletal maturity and was chosen to potentially maximize the impact of sActRIIB-mFc treatment on musculoskeletal development.<sup>33</sup> The 10mg/kg dose of sActRIIB-mFc was previously found

to significantly increase muscle mass, which was not further increased by a 30mg/kg dose.<sup>28</sup> Animals were not randomized into treatment groups and the investigators were not blinded as to the identity of the animals. At 4 months of age, mice were anesthetized to evaluate hindlimb skeletal muscle contractility *in situ*, and then euthanized, and the hindlimb skeletal muscles were harvested for histologic evaluation, cross sectional area measurement, and citrate synthase activity.

### Contractile Properties

Contractile properties of the soleus (Sol), gastrocnemius (Gast), and tibialis anterior (TA) muscles in male and female WT, *+G610C*, and *oim/oim* mice treated with either vehicle or sActRIIB-mFc were determined as previously described.<sup>12</sup> Briefly, mice were anesthetized and the left Sol, Gast, and TA muscles were surgically exposed at their distal insertions. The distal tendon of each muscle was attached to the Grass force transducer and sequentially tested. The distal tendon was adjusted in length so that passive tension was zero grams. The sciatic nerve was isolated and placed on a stimulating electrode and a twitch was obtained as previously described.<sup>12</sup> 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.<sup>34</sup> All data were collected using Power Lab® (ADInstruments, Colorado Springs, CO), and analyzed blind to genotype, sex, and treatment.

### Tissue Harvest

Following the contractile function testing, the left (stimulated) and right Sol, plantaris (Plant), Gast, TA, and quadriceps (Quad) muscles were removed, cleaned of extraneous tissue, blotted, and weighed. Left Sol, Gast, and TA muscles were then placed in 4% paraformaldehyde for 24 hours and then transferred to 70% ethanol for future staining with hematoxylin and eosin for morphologic evaluation. The right side Gast was snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for future quantitation of citrate synthase activity.

### Histologic Evaluation and Cross-Sectional Myofiber Measurements

Left-sided paraformaldehyde and ethanol prepared muscles were transversely sectioned at the middle of the muscle belly, embedded in paraffin and then sectioned at  $5\mu\text{m}$  and stained with hematoxylin and eosin (H&E).<sup>12</sup> An average of 300 myofibers was evaluated for morphology and evidence of damage or inflammation, and their myofiber cross-sectional areas were determined using Image J Software (NIH).<sup>12</sup> Analyses were performed blind to genotype, sex, and treatment

### Citrate Synthase Activity

Citrate synthase was determined in mixed gastrocnemius muscle as a measure of mitochondrial content/function using the methods of Srere et al. as previously described<sup>35,36</sup>.

### Locomotor Activity

Locomotor activity of WT and *oim/oim* mice was monitored using Med Associates Open Field Test Environment monitors (Georgia, VT) at 1, 2, 3 and 4 months of age as described

previously.<sup>18</sup> The Med Associates' Open Field Activity Software was used to evaluate the number of recorded sensor breaks and converting the data into distance traveled (cm). Mice were placed individually into the chamber for 60 minutes per day for five consecutive days. The mice were acclimated on first 2 days and the data from days 3–5 was summated and used in the statistical analyses.

## Statistical Analyses

Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC). Data were analyzed as a 3×2×2 factorial [3 genotypes, 2 sexes and 2 treatments (vehicle and sActRIIB-mFc treated)] using Fisher's Protected Least Significant Difference.<sup>37,38</sup> Locomotor activity data was analyzed as a 2×2×4 factorial [2 genotypes, 2 sexes, and 4 time points]. Genotype and sex interactions and the genotype and sex main effects were evaluated. Body, muscle and relative muscle weights (Gast, TA, and Sol), muscle contractile function (Gast and Sol), muscle myofiber cross sectional area (Gast and Sol), and activity levels exhibited a main genotype effect, allowing pooling of genotype-matched male and female values in the absence of genotype and sex interactions. The main genotype effect values for these parameters are presented as the LSmeans±SE. Genotype and sex interactions were present only for the TA myofiber cross-sectional area and contractile generating force. Supplement Tables 1–4 contain the body, muscle and relative muscle weights, muscle contractile function, and muscle myofiber cross sectional area means ± SE separated by sex, treatment, and genotype. If heterogeneous variations were present, a log transformation was used to stabilize the variation. If the log transformation failed to stabilize the variation, a nonparametric ranked analysis was performed according to Conover et al.<sup>39</sup> Differences were considered to be significant at  $p < 0.05$ .

## Results

### Body Weights

Vehicle treated *oim/oim* mice had lower body weights as compared to both WT and *+/-G610C* counterparts, while sex- and treatment-matched *+/-G610C* and WT mice were equivalent (Figure 1). Mice treated with sActRIIB-mFc exhibited differences in body weights after 1-week of treatment (after the 2<sup>nd</sup> injection) as compared to vehicle treated control mice regardless of genotype and sex (Figure 1). At 4 months of age, sActRIIB-mFc treated male and female mice had 20% and 24% increases in body weight, respectively, as compared to their vehicle treated counterparts regardless of genotype (Figure 1).

### Muscle Weights

The hindlimb skeletal muscles [soleus (Sol), plantaris (Plant), gastrocnemius (Gast), tibialis anterior (TA), and quadriceps (Quad)] of the vehicle treated *oim/oim* mice exhibited reduced muscle wet weights as compared to both WT and *+/-G610C* counterparts, while there were no differences in the hindlimb muscle weights of WT and *+/-G610C* mice (Figure 2, Supplement Table 1&2). Mice treated with sActRIIB-mFc exhibited increases in the muscle wet weights of all five hindlimb skeletal muscles as compared to their vehicle treated counterparts regardless of genotype (Figure 2). The effects of genotype and sActRIIB-mFc treatment were still evident when muscle masses were normalized to their body weights

[relative muscle weights (muscle wet weight/body weight)], except for sActRIIB-mFc treated Sol muscles for the three genotypes and Gast muscle for WT and *oim/oim* mice (Figure 3, Supplement Table 1&2). When muscle masses were normalized to their femur length, the sActRIIB-mFc treatment effects were still evident in all genotypes regardless of muscle groups, suggesting the increase in body weight is due to increases in muscle mass (data not shown).

### Muscle Histology and Myofiber Cross-Sectional Area

Histological evaluation found no evidence of necrosis, degeneration, or regeneration in vehicle or sActRIIB-mFc treated WT, *+G610C*, and *oim/oim* mouse skeletal muscle morphology (Figure 4A–F, Supplement Table 3&4). sActRIIB-mFc treated WT and *+G610C* mice myofiber cross-sectional areas (CSA) were increased in the Sol, Gast and TA muscles as compared to their vehicle treated counterparts ([Sol WT: 22% and *+G610C*: 21%] & [Gast WT: 20% and *+G610C*: 26%] & [TA Male WT: 59%, Male *+G610C*: 59%, Female WT: 35%, and Female *+G610C*: 12%]) (Figure 4G–I). For the *oim/oim* mice, only the female *oim/oim* TA muscle exhibited increased myofiber CSA in response to sActRIIB-mFc treatment (Figure 4I).

### Muscle Contractile Generating Capacity

The Sol, Gast, and TA muscles of *+G610C* mice exhibited equivalent levels of absolute whole muscle contractile generating capacity [peak tetanic force ( $P_o$ ;g)], relative contractile generating capacity [relative  $P_o$ ; peak tetanic force ( $P_o$ ;g)/muscle weight (mg)], and specific contractile generating capacity [specific  $P_o$ ; peak tetanic force ( $P_o$ ;g)/muscle myofiber cross sectional area ( $\mu\text{m}^2$ )] as compared to their WT counterparts regardless of sActRIIB-mFc treatment except for the male relative and specific  $P_o$  of TA muscle and pooled specific  $P_o$  of Gast muscle (Figure 5 and 6, Supplement Table 3&4). Vehicle treated *oim/oim* mice had reduced peak tetanic force in Sol, Gast, and TA muscles, and reduced relative  $P_o$ , and specific  $P_o$  in Gast, and TA muscles as compared to their WT counterparts (Figure 5 and 6). sActRIIB-mFc treated WT and *+G610C* mice had 22% and 24% increase in Sol  $P_o$  and 11% and 5% increase in Gast  $P_o$ , respectively, although they did not reach significance. sActRIIB-mFc treated *oim/oim* mice exhibited 41% (Sol muscle), 35% (Gast muscle), 43% (male TA muscle) and 27% (female TA muscle) increase in peak tetanic force as compared to vehicle treated counterparts (Figure 5). When normalized to muscle size the relative  $P_o$  of the Sol muscle in the *oim/oim* mouse was still greater with sActRIIB-mFc treatment (Figure 5). Interestingly, the relative  $P_o$  of the Gast and TA muscles were decreased in sActRIIB-mFc treated WT and *+G610C* mice as compared to their vehicle treated counterparts (Figure 5). The specific  $P_o$  of the TA muscles were decreased in male WT and *+G610C* mice, and the specific  $P_o$  in Gast muscle was decreased in *+G610C* mice with sActRIIB-mFc treatment as compared to their vehicle treated counterparts (Figure 6).

### Citrate Synthase Activity

*Oim/oim* Gast muscle exhibited a 16% increase in citrate synthase activity as compared to WT counterparts, where the levels of *+G610C* Gast synthase activity were equivalent to their WT counterparts (Figure 7). There was no change in citrate synthase activity with sActRIIB-mFc treatment regardless of genotype.

## Locomotor Activity Levels

*Oim/oim* mice had reduced locomotor activity levels at 1, 2, and 4 months old as compared to WT mice (Figure 8).

## Discussion

Consistent with the previous studies, *oim/oim* mice exhibited reduced body weights, muscle mass and contractile function compared to WT littermates, whereas the *+G610C* mice did not exhibit any muscle pathology (Figure 1, 2, and 5).<sup>12,18</sup> Similar to our findings in OI mouse models, several clinical studies of muscle function found that individuals with type I, III, and IV OI exhibited decreased ambulation with reduced muscle strength, and exercise capacity.<sup>14–17,40,41</sup> Brizola et al. suggested that the level of ambulation and muscle function in OI were inversely correlated with the severity of bone deformity regardless of OI types.<sup>17</sup> Palomo et al. reported reduced transverse forearm muscle cross-sectional area in OI type I and III patients, but not in type IV patients; however there was no functional data.<sup>41</sup> Interestingly, *+G610C*, which models type IV human OI, did not exhibit a muscle pathology or differences in activity levels relative to WT mice.<sup>18</sup> This could reflect species differences and/or mutation specific effects.

Our findings demonstrate an increase in body size and hindlimb skeletal muscle weights with systemic administration of sActRIIB-mFc in WT, *+G610C*, and *oim/oim* mice, potentially due to increased myofiber CSA, suggesting that inhibition of myostatin via sActRIIB-mFc is effective in increasing muscle mass regardless of the presence or absence of OI muscle pathology. Increased muscle mass was associated with increased absolute muscle contractile function without altered relative and specific muscle contractile function in sActRIIB-mFc treated *oim/oim* mice. Similarly, DiGirolamo and colleagues showed increased muscle mass with 4 weeks administration of soluble activin receptor IIB in *oim/oim* mice, although the increases in limb muscle weights did not always reach significance and were not as great as the impact on pectoralis muscle.<sup>22</sup> We demonstrated increases in all five hindlimb skeletal muscles weights with sActRIIB-mFc treatment. The difference in magnitude of the response likely reflects the duration and frequency of treatment (once per week for 4 weeks vs. bi-weekly for 8 weeks) and the age of treatment initiation (12 weeks vs. 8 weeks of age). 12 week old mice are adult mice near skeletal maturity, whereas 8 week old mice are still within a more rapid growth period and may be more responsive to sActRIIB-mFc treatment. Additionally, older *oim/oim* mice may have experienced fractures (possibly an accumulation of fractures) as compared to younger *oim/oim* mice, with these fractures potentially decreasing mobility and impacting muscle mass and function.

Increased muscle mass with sActRIIB-mFc treatment was also concomitant with increased myofiber CSA; with sActRIIB-mFc treated mice exhibiting increased Sol, Gast, and TA myofiber CSA as compared to vehicle treated mice regardless of sex and genotype, although *oim/oim* mice did not reach significance (Figure 4). Previous studies in dystrophic muscle mouse models have also consistently reported increases in muscle mass and myofiber CSA.<sup>23–26,28</sup> Interestingly, MacDonald et al. reported that muscle atrophy in mice due to surgical denervation was not protected by soluble ActRIIB treatment, suggesting increased

muscle mass and force via myostatin inhibition may not be effective when there is deterioration of the neuro-muscular junction.<sup>42</sup> Muscle atrophy and/or weakness in OI has generally been attributed to immobilization, secondary impacts of neurological impairment or orthopedic casting, and prolonged bedrest due to fractures.<sup>43</sup>

To determine if the increase in muscle mass and myofiber CSA were associated with improved muscle function, we evaluated the contractile generating force of the Sol, Gast, and TA muscles. Surprisingly, sActRIIB-mFc treated *oim/oim* mice exhibited increased absolute whole muscle contractile force in Sol, Gast, and TA muscles as compared to vehicle treated counterparts, while WT and *+G610C* showed only increasing trends (Figure 5). Although other studies of mouse models with muscle atrophy have shown soluble ActRIIB treatment increased absolute muscle contractile function of compromised muscle,<sup>23,24,28,44</sup> there were also reports of earlier muscle fatigability,<sup>26,45</sup> decreased gene expression of components involved in mitochondrial function and oxidative phosphorylation,<sup>46,47</sup> as well as reduced myofiber specific function in *mdx* mice (mouse model of Duchenne muscular dystrophy) mice with treatment of higher concentrations of soluble ActRIIB.<sup>25</sup> Consistent with these studies, myostatin knock-out (*MSTN<sup>-/-</sup>*) mice exhibit centralized nuclei, a sign of muscle degenerations, and reduced relative muscle contractile function as compared to WT and heterozygous myostatin deficient (*MSTN<sup>+/-</sup>*) littermates,<sup>48</sup> suggesting the complete absence of myostatin is detrimental to muscle myofiber function.

Our results demonstrate sActRIIB-mFc treated *oim/oim* mice were able to produce higher or equivalent levels of relative and specific muscle contractile forces as compared to vehicle treated counterparts, while WT and *+G610C* mice exhibited decreases in relative and specific muscle contractile forces in their Gast and TA muscles. The percent decrease of the relative contractile force of sActRIIB-mFc treated WT and *+G610C* Gast and TA muscles [Male WT: -23% (Gast) and -30% (TA); Male *+G610C*: -36% (Gast) and -43% (TA) (Supplement Table 3)] were greater relative to age-matched 4 month old heterozygous *MSTN<sup>+/-</sup>* mice [Male: -18% (Gast) and -30% (TA)],<sup>48</sup> but not as robust as myostatin knockout (*MSTN<sup>-/-</sup>*) mice [Male: -39% (Gast) and -39% (TA)].<sup>48</sup> These data suggest that sActRIIB-mFc treatment (bi-weekly, 10mg/kg) may reduce available myostatin levels to below that present in *MSTN<sup>+/-</sup>* mice. Unlike *MSTN<sup>-/-</sup>* mice, heterozygous *MSTN<sup>+/-</sup>* had increased muscle mass with mild lesions without exhibiting a dramatic decrease in relative contractile function.<sup>48</sup> Consistent with the reported *MSTN<sup>+/-</sup>* findings, whippet dogs heterozygous for a myostatin mutation exhibit better racing performance than normal whippet dogs or whippet dogs homozygous for the *MSTN* defect.<sup>49</sup> These data suggest that the magnitude of myostatin inhibition is critical to muscle function and exercise performance.

The observed improvement of *oim/oim* muscle contractile function with ActRIIB-mFc treatment, not seen in WT or *+G610C* muscle may be due to inherent differences in the basal levels of muscle function. Children with OI can experience multiple fractures that can hamper their daily activity leading to hypoactivity and muscle atrophy. Van Brussel et al. reported greater degrees of improvement in muscle force and aerobic capacity in OI type I and IV children with well-tailored physical training as compared to healthy individuals.<sup>40</sup> Analogous to human OI individuals, hypoactivity in *oim/oim* mice can contribute to muscle



weakness. The lower basal level muscle strength of *oim/oim* mice may have greater capacity to respond to myostatin inhibition as reflected by the increase in muscle contractile function with ActRIIB-mFc treatment relative to WT and *+G610C* mice.

Physical exercise contributes to bone health either directly or indirectly via muscle loads during prepubertal/pubertal growth in healthy children; a 6-year of exercise program in healthy children demonstrated improved bone geometry with increased bone mass.<sup>50</sup> However, OI children often experience reduced physical activity levels and exhibit decreased exercise tolerance as compared to healthy age-matched individuals.<sup>15,51</sup> This has been postulated to be associated with pain and/or behavioral choices due to concerns of fracture.<sup>52,40</sup> Similarly, *oim/oim* mice exhibit reduced activity levels, which in addition to muscle weakness may reflect complications due to skeletal deformity or activity-associated pain. Although we have not addressed the impact of pain in *oim/oim* mice, in another OI mouse model, the *Col1a1<sup>Jrt/+</sup>* mice report hypersensitivity to mechanical, heat and cold stimuli, reduced locomotor activity, and increased limping.<sup>53</sup>

Treatment with sActRIIB-mFc resulting in improved muscle function may also facilitate motor function of *oim/oim* mice, which may then allow development of tailored exercise regimens to further improve both the muscle and bone. The *mdx* mouse is characterized by reduced aerobic metabolism and muscle wasting. The combination of exercise and administration of sActRIIB-mFc to the *mdx* mice was more effective in reconditioning the skeletal muscle gene expression profile to reflect healthy WT mouse muscle than either exercise or sActRIIB-mFc treatment alone.<sup>54</sup> In addition, further improvement in muscle and bone properties has been shown with a combination of myostatin deficiency and exercise in *MSTN<sup>-/-</sup>* mice.<sup>55</sup> In a separate study, neutralizing myostatin antibody- treated 24 month old mice undergoing treadmill exercise had demonstrated improved exercise performance with increased habitual ambulatory activity relative to either antibody treatment or treadmill exercise alone.<sup>56</sup>

Several studies raise concerns about impairment of mitochondrial function with myostatin inhibition. A limited group of studies in *MSTN<sup>-/-</sup>* mice have demonstrated increased force production that was not proportional to their muscle hypertrophy, which may reflect decreased mitochondrial function,<sup>45-47,57</sup> muscle fiber type switching,<sup>57,58</sup> deficits in oxidative metabolism that can lead to decreased endurance capacity,<sup>57-60</sup> and/or decreased capillary density in muscle that alters the aerobic metabolism.<sup>58</sup> Unlike *MSTN<sup>-/-</sup>* mice, Bechir and colleagues have shown energy status and function of glycolytic muscles were not disturbed during 6 min fatigue exercise with ActRIIB blockade in both WT and *mdx* mice.<sup>44</sup> We examined citrate synthase activity, a measure of mitochondrial content and function, in the mixed Gast muscle and found that citrate synthase activity was not altered by sActRIIB-mFc treatment for all genotypes. This suggests blockade of myostatin via sActRIIB-mFc does not alter this marker of mitochondrial function in Gast muscles. Interestingly, *oim/oim* Gast muscle had greater citrate synthase activity than WT or *+G610C* Gast muscle (+16%), which may reflect compensatory up-regulation of *oim/oim* mitochondrial function to offset its compromised muscle function.

In summary, muscle weakness is often observed in individuals with OI and clinicians encourage physical exercise to strengthen both the muscle and bone properties. Depending on clinical severity, individuals with OI may be limited in their options for exercise. Our study demonstrates that sActRIIB-mFc treated *oim/oim* mice, which model the moderately severe type III OI in humans, exhibited increases in muscle mass with improved contractile function without any apparent adverse effects, while the WT and *+G610C* mice treated with sActRIIB-mFc exhibited compromised muscle function. Further investigation of the underlying mechanisms responsible for the improved muscle function in *oim/oim* mice undergoing sActRIIB-mFc treatment and the differential response of WT and *+G610C* mice remains to be evaluated. This study provides new insight and potential clues to design new approaches to improve muscle and/or bone properties in OI.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

### OI

Osteogenesis Imperfecta

### WT

Wildtype

### *oim*

Osteogenesis imperfecta murine

### vehicle [TBS]

Tris-Buffered Saline

### sActRIIB-mFc

Soluble activin receptor type IIB

### IP

Intraperitoneal

### Sol

Soleus

**Plant**

Plantaris

**Gast**

Gastrocnemius

**TA**

Tibialis Anterior

**Quad**

Quadriceps

**CSA**

Cross sectional area

**peak tetanic force,  $P_0$**

Absolute whole muscle contractile generating capacity

**relative  $P_0$**

Relative contractile generating capacity

**specific  $P_0$**

Specific contractile generating capacity

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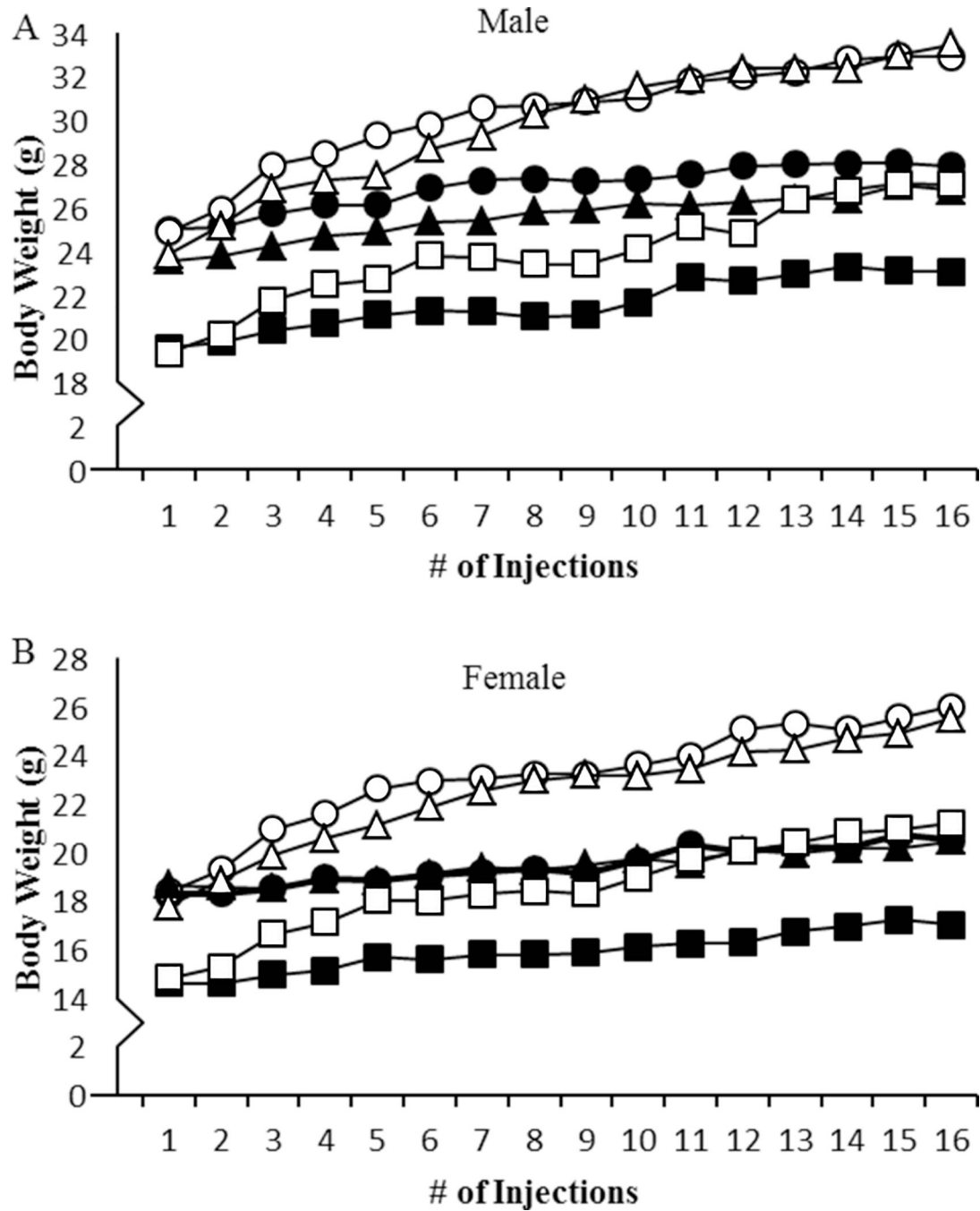
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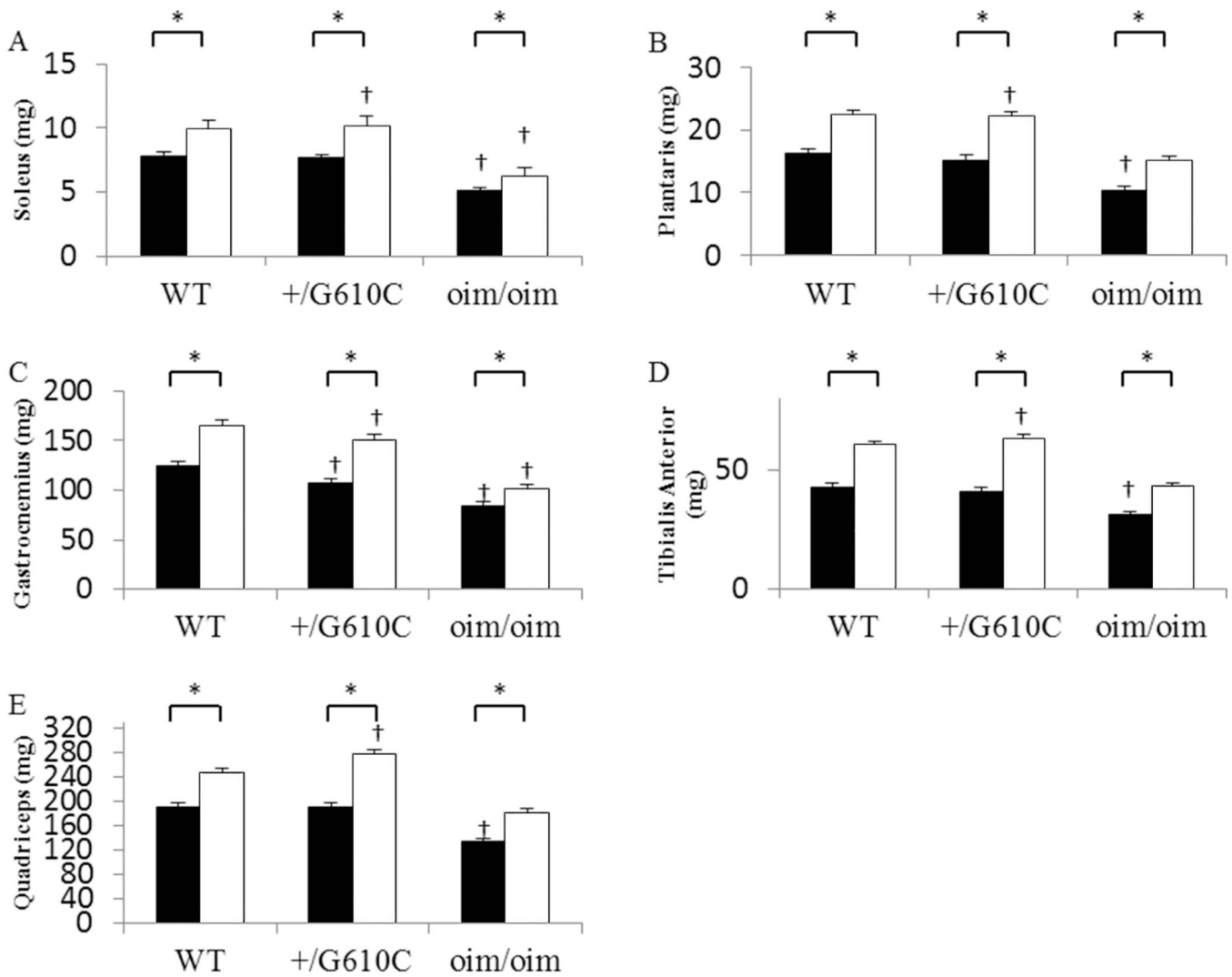
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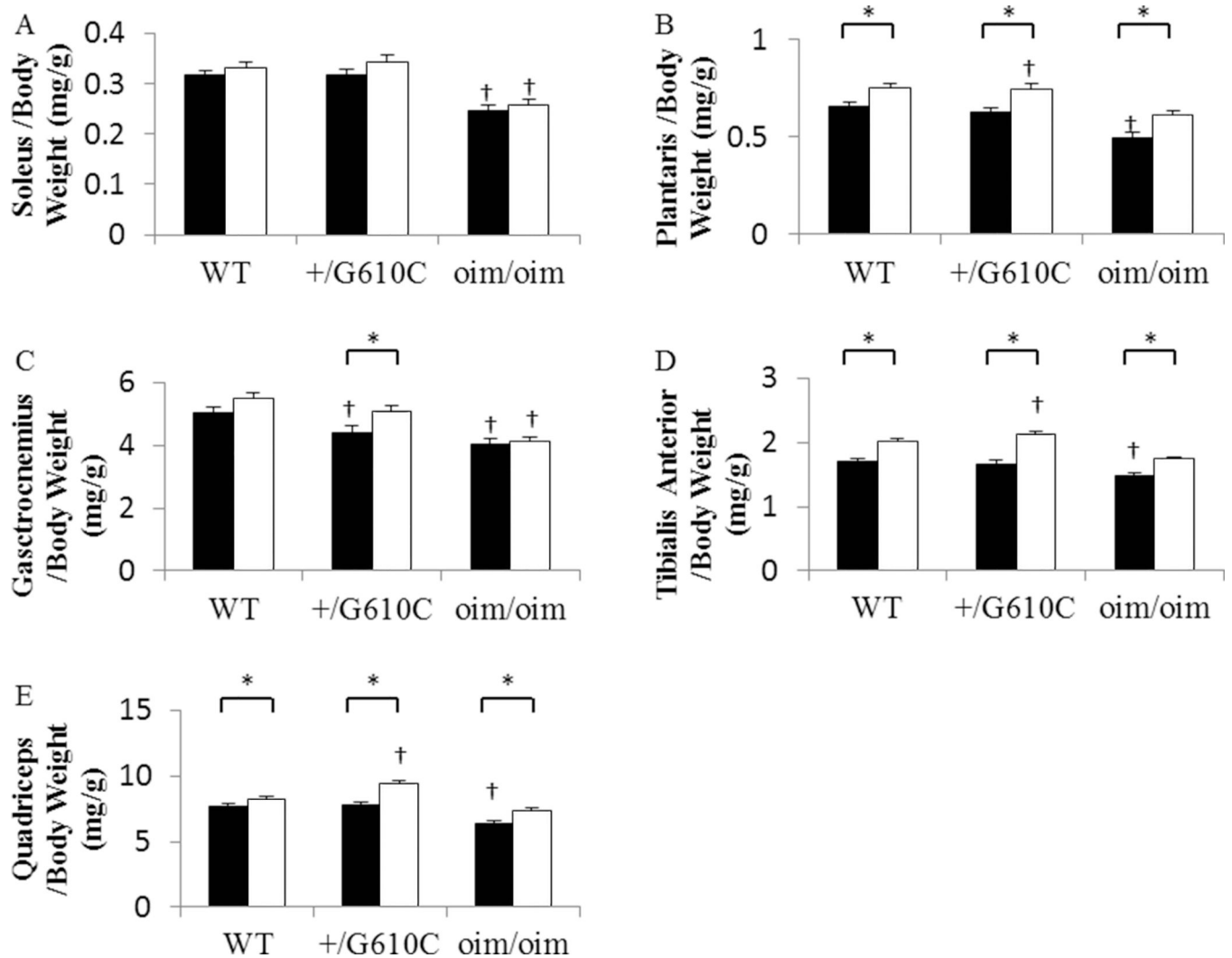


**Figure 1.** sActRIIB-mFc treated mice (*open symbols*) had increased body weight throughout the study as compared to vehicle treated counterparts (*solid symbols*) regardless of sex and genotype. Differences between sActRIIB-mFc and vehicle treatments were observed by the 3<sup>rd</sup> injection regardless of genotype and sex. A) Male and B) female WT (*circle*), *+/G610C* (*triangle*), and *oim/oim* (*square*). (n=8–14 per groups)

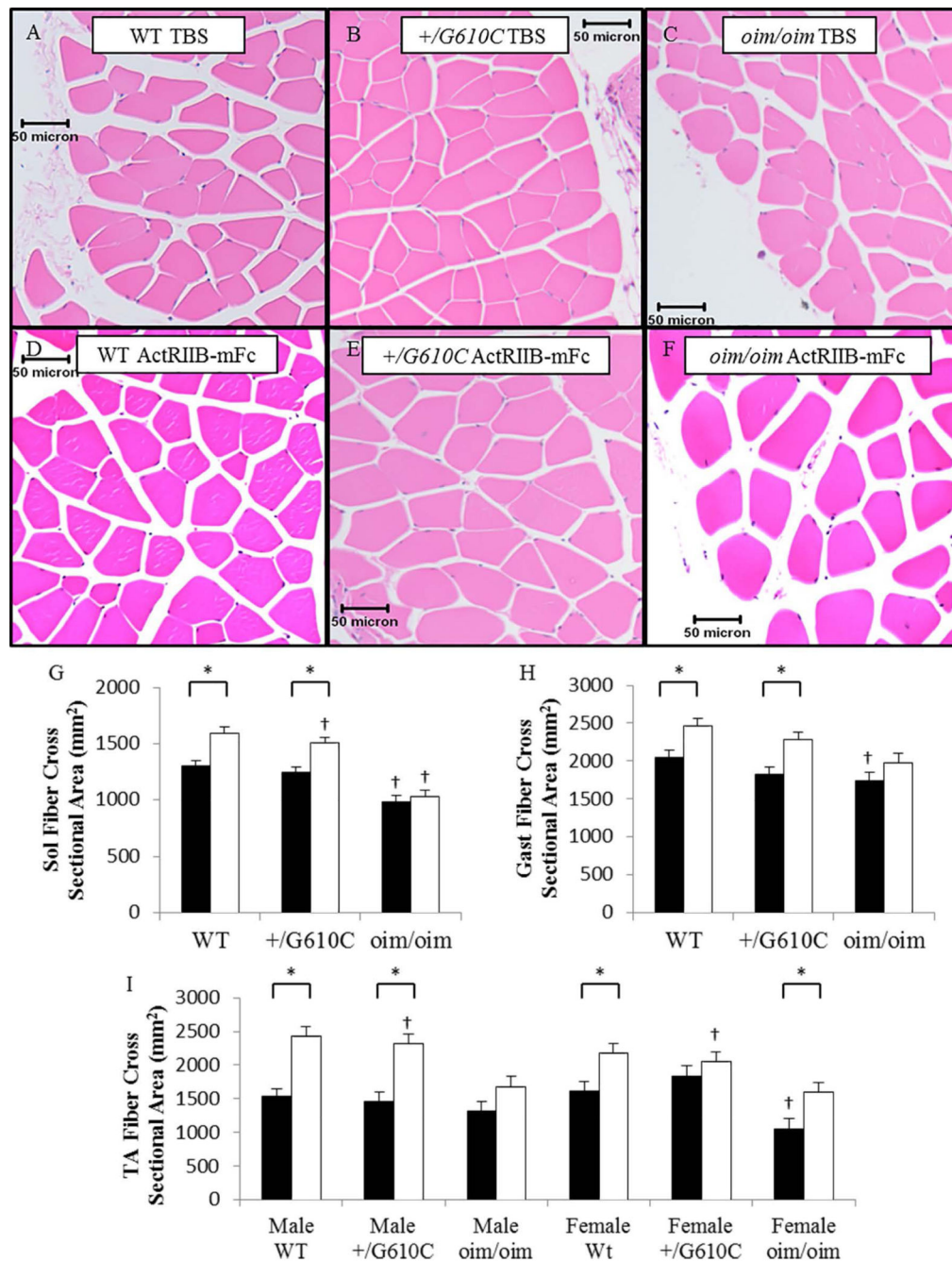




**Figure 2.** sActRIIB-mFc treated mice (*open bars*) had increased hindlimb skeletal muscle weights as compared to vehicle treated counterparts (*solid bars: control*) regardless of genotype. (A) Soleus, (B) Plantaris, (C) Gastrocnemius, (D) Tibialis Anterior, and (E) Quadriceps muscle wet weights of 4 months old WT, +/G610C, and oim/oim (n=16–25 per groups). The genotype main effect was evaluated. Values are the LSmeans±SE of the combined genotype values regardless of sex. \* $p < 0.05$ ; † $p < 0.05$  vs. WT+vehicle treated mice.



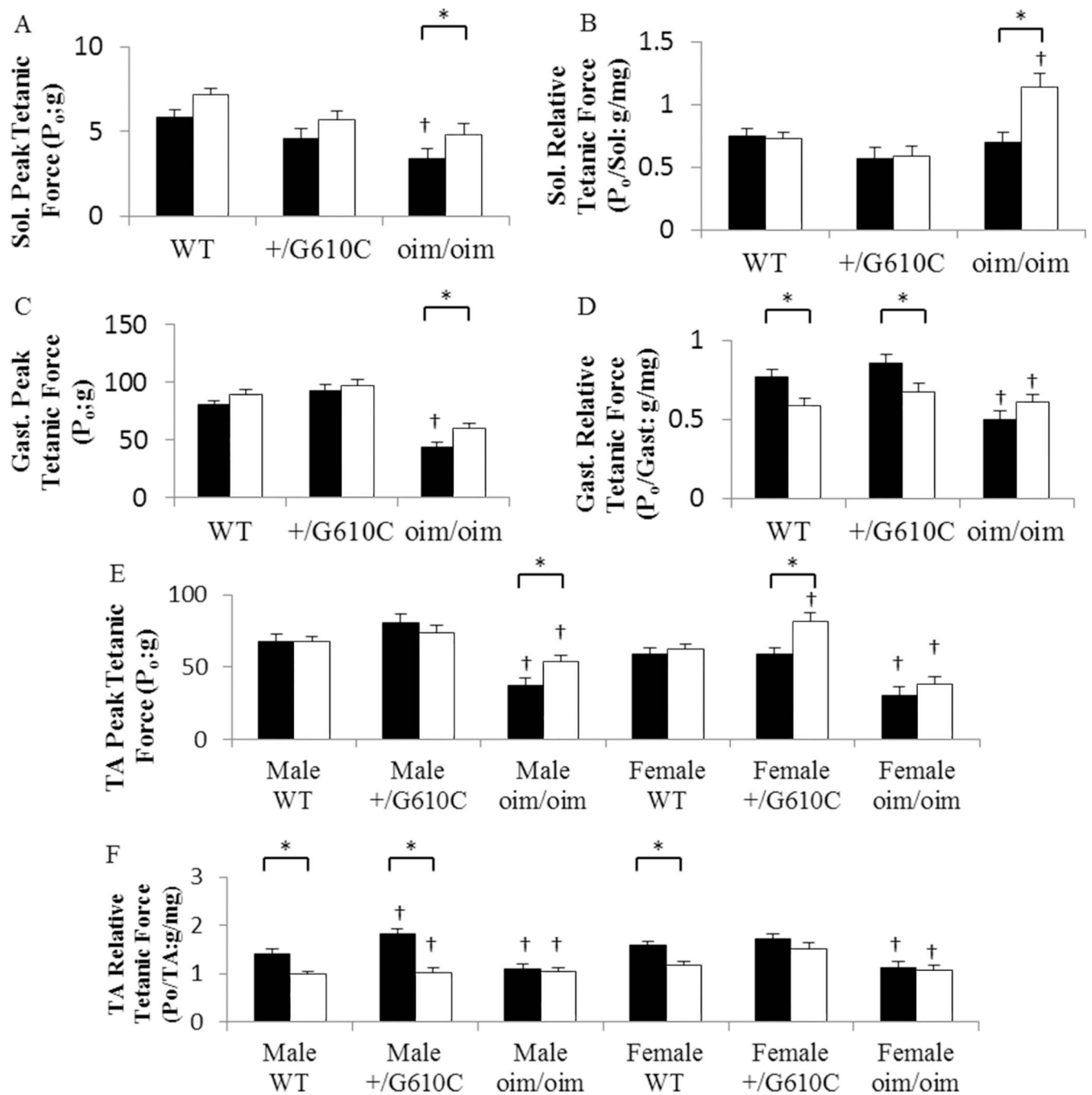
**Figure 3.** sActRIIB-mFc treated mice (*open bars*) had increased relative skeletal muscle weights as compared to vehicle treated counterparts (*solid bars: control*) regardless of genotypes. (A) Soleus/Body Weight, (B) Plantaris/Body Weight, (C) Gastrocnemius/Body Weight, (D) Tibialis Anterior/Body Weight, and (E) Quadriceps/Body Weight of 4 months old WT, +/G610C, and oim/oim (n=16–25 per groups). The genotype main effect was evaluated. Values are the LSmeans±SE of the combined genotype values regardless of sex. \* $p < 0.05$ ; † $p < 0.05$  vs. WT+vehicle treated mice.



**Figure 4.**

Hematoxylin and eosin (H&E) stained muscle myofiber cross-sections of the gastrocnemius (Gast) muscle from 4 month old female mice (A–F). There was no evidence of degeneration, regeneration, or multiple centralized nuclei, 10× magnification. sActRIIB-mFc treated mouse myofiber cross-sectional areas (CSA) (*open bars*) were larger than vehicle treated counterparts (*solid bars*) (G–I). *oim/oim* mice exhibited reduced average muscle myofiber CSA as compared to WT and *+/G610C* counterparts. sActRIIB-mFc treated WT and *+/G610C* mice exhibited increased muscle myofiber CSA as compared to vehicle treated

counterparts. (G) Sol myofiber CSA, (H) Gast myofiber CSA, (I) TA myofiber CSA (n=9–12 per groups; TA muscle groups are n=4–7). The genotype main effect was evaluated. Gast and Sol values are the LSmeans±SE of the combined genotype values regardless of sex, and the TA exhibited a genotype × sex interaction, and the values are LSmeans±SE separated by sex and genotype. \* $p<0.05$ ; † $p<0.05$  vs. WT+vehicle treated mice.



**Figure 5.** *oim/oim* mice had reduced absolute and relative peak tetanic force ( $P_0$ ) as compared to WT and *+G610C* counterparts (A–F). sActRIIB-mFc treated *oim/oim* mice exhibited increased peak  $P_0$  as compared vehicle treated counterparts (A, C and E). sActRIIB-mFc treated WT and *+G610C* mice exhibited reduced relative peak tetanic force as compared to vehicle treated counterparts (B, D, and F). (A) Sol. Peak tetanic force ( $P_0$ ), (B) Sol. relative  $P_0$ , (C) Gast Peak  $P_0$ , (D) Gast. Relative  $P_0$ , (E) TA Peak  $P_0$ , and (F) TA relative  $P_0$  of 4 months old WT, *+G610C*, and *oim/oim* vehicle (solid bars: control) and sActRIIB-mFc (open bars)

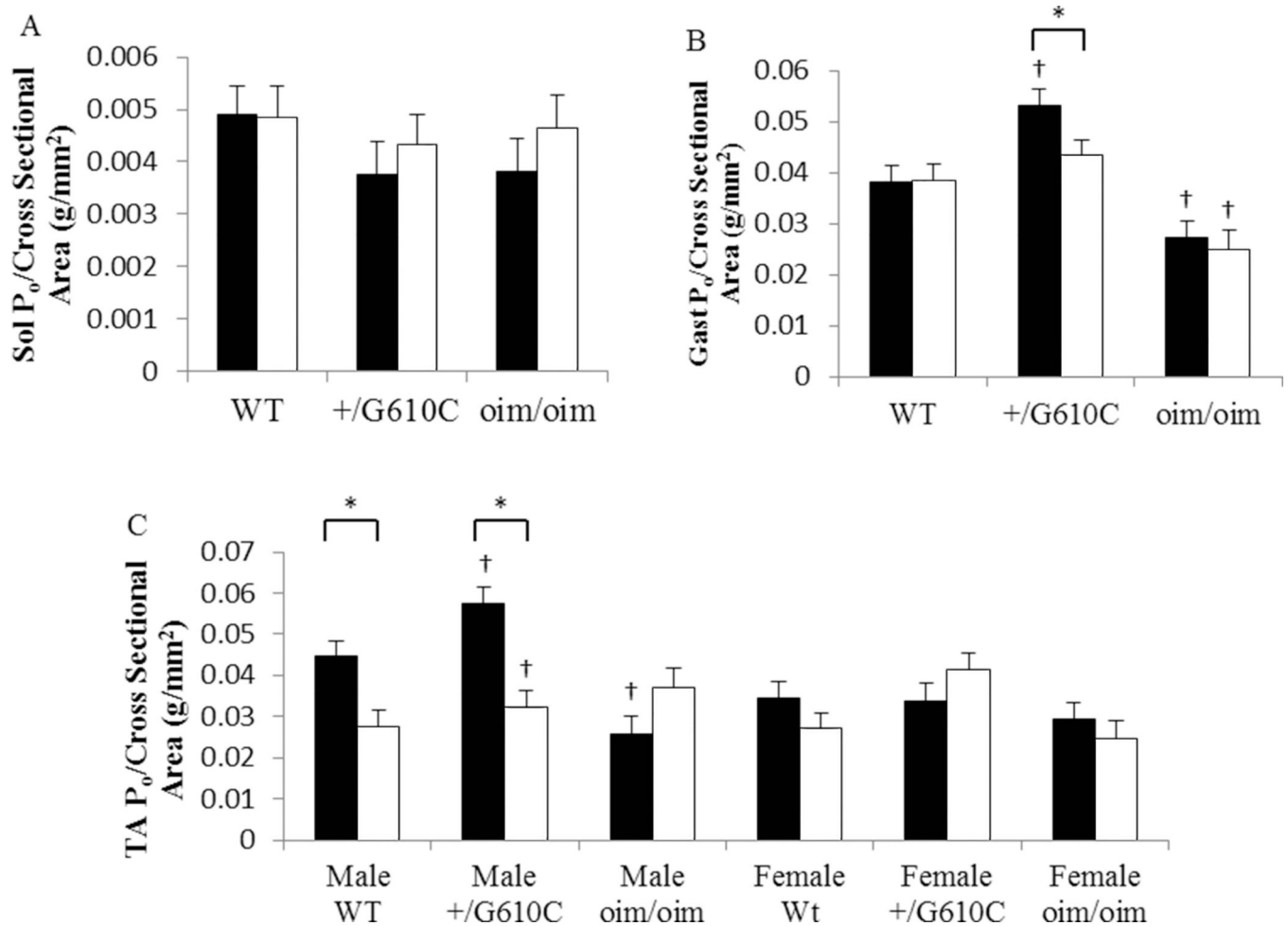
treated mice. (n=10–30 per groups; TA Peak P<sub>0</sub> n=6–16 per groups). The genotype main effect was evaluated. Gast and Sol values are the LSmeans±SE of the combined genotype values regardless of sex, and the TA exhibited a genotype × sex interaction, and the values are LSmeans±SE separated by sex and genotype. \* $p < 0.05$ ; † $p < 0.05$  vs. WT+vehicle treated mice.

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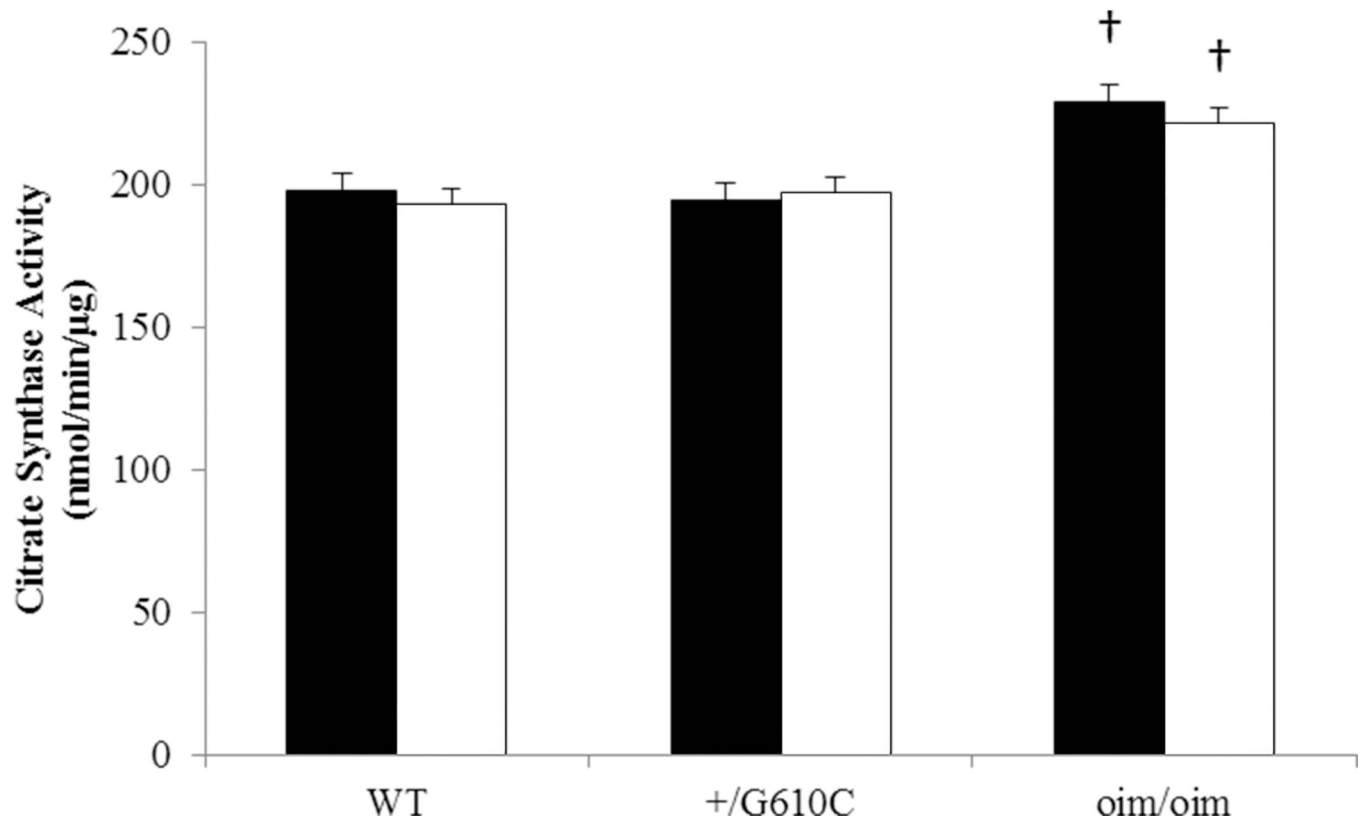
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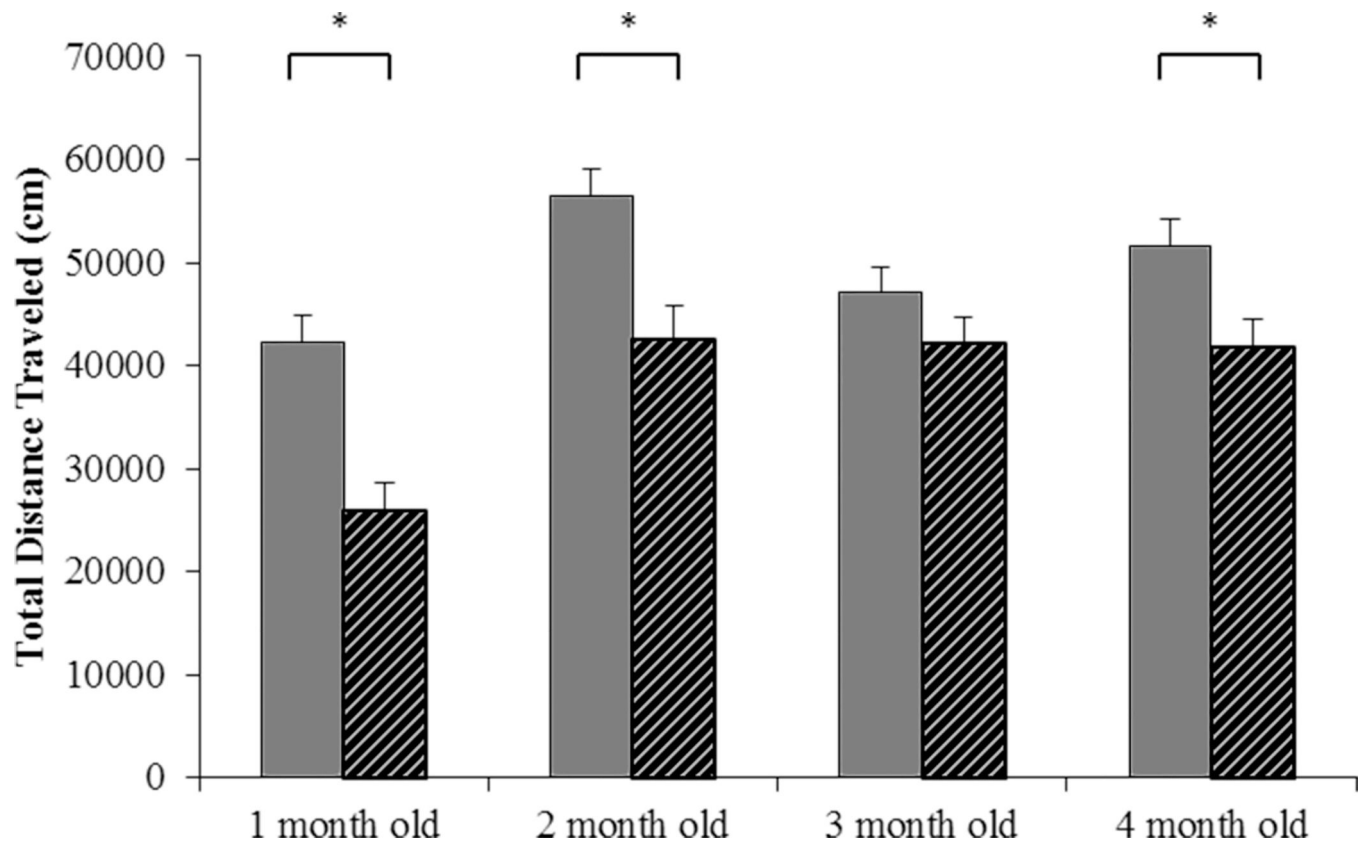


**Figure 6.** sActRIIB-mFc treated *+G610C* mice Gast muscle had reduced specific tetanic force [specific P<sub>0</sub> (Peak P<sub>0</sub>/Muscle myofiber CSA)] as compared to vehicle treated counterparts (B). sActRIIB-mFc treated male WT and *+G610C* mice TA muscle had reduced specific P<sub>0</sub> as compared to vehicle treated counterparts (C). (A) Sol Specific P<sub>0</sub>, (B) Gast Specific P<sub>0</sub>, and (C) TA Specific P<sub>0</sub> of 4 months old WT, *+G610C*, and *oim/oim* vehicle (*solid bars: control*) and sActRIIB-mFc (*open bars*) treated mice. (n=9–12 per groups; TA muscle groups are n=4–7). The genotype main effect was evaluated. Gast and Sol values are the LSmeans±SE of the combined genotype values regardless of sex, and the TA exhibited a genotype × sex interaction, and the values are LSmeans±SE separated by sex and genotype. \**p*<0.05; †*p*<0.05 vs. WT+vehicle treated mice.



**Figure 7.** *oim/oim* mice Gast muscle had increased citrate synthase activity as compared to WT mice. (n=10–12 per groups). There was no difference in citrate synthase activity between vehicle (*solid bars: control*) and sActRIIB-mFc (*open bars*) treated mice regardless of genotype. The genotype main effect was evaluated. Values are LSmeans±SE of the combined genotype values regardless of sex.  $p < 0.05$ ; <sup>†</sup> $p < 0.05$  vs. WT+vehicle treated mice.





**Figure 8.** *oim/oim* mice (*grey stripe*) traveled a reduced distance (cm) over a 1 hour period on 3 consecutive days (3 hours of total travel time) as compared to WT (*grey bars*) littermates at 1, 2, and 4 months old. (n= 16–25 per groups). The genotype main effect was evaluated. Values are LSmeans±SE of the combined genotype values regardless of sex. \* $p < 0.05$