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Authors

Kim, Jung A
Roy, Roland R
Zhong, Hui
[et al.](#)

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PPAR δ PRESERVES A HIGH RESISTANCE TO FATIGUE IN THE MOUSE MEDIAL GASTROCNEMIUS AFTER SPINAL CORD TRANSECTION

Jung A. Kim, Ph.D.^{1,*}, Roland R. Roy, Ph.D.¹, Hui Zhong, M.D.¹, William A. Alaynick, Ph.D.⁵, Emi Embler, M.S.⁵, Claire Jang, B.S.¹, Gabriel Gomez, B.S.¹, Takuma Sonoda, B.S.¹, Ronald M. Evans, Ph.D.^{5,6}, and V. Reggie Edgerton, Ph.D.^{1,2,3,4}

¹Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA 90095

²Department of Neurobiology, University of California, Los Angeles, Los Angeles, CA 90095

³Department of Neurosurgery, University of California, Los Angeles, Los Angeles, CA 90095

⁴Brain Research Institute, University of California, Los Angeles, Los Angeles, CA 90095

⁵Gene Expression Laboratory, Salk Institute, La Jolla CA 92037

⁶Howard Hughes Medical Institute, La Jolla, CA 92093

Abstract

Introduction—Skeletal muscle oxidative capacity decreases and fatigability increases after spinal cord injury. Transcription factor peroxisome proliferator-activated receptor δ (PPAR δ) promotes a more oxidative phenotype.

Methods—We asked whether PPAR δ overexpression could ameliorate these deficits in the medial gastrocnemius (MG) of spinal cord transected (ST) adult mice.

Results—Time-to-peak tension and half-relaxation times were longer in PPAR δ -Con and PPAR δ -ST compared to littermate wild-type (WT) controls. Fatigue index was 50% higher in PPAR δ -Con than WT-Con and 70% higher in the PPAR δ -ST than WT-ST. There was an overall higher percent of darkly stained fibers for succinate dehydrogenase in both PPAR δ groups.

Discussion—The results indicate a conversion towards slower, more oxidative, and less fatigable muscle properties with overexpression of PPAR δ . Importantly, the elevated fatigue resistance was maintained after ST, suggesting that enhanced PPAR δ expression, and possibly small molecule agonists, could ameliorate the increased fatigability routinely observed in chronically paralyzed muscles.

Send correspondence to: V. Reggie Edgerton, Ph.D., Department of Integrative Biology and Physiology, 610 Charles E. Young Drive East, Los Angeles, CA 90095-7239, Telephone: (310) 825-1910, FAX: (310) 933-1965, vre@ucla.edu.

*Current address: Department of Exercise Science, University of Puget Sound, Tacoma, WA 98416

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INTRODUCTION

Most skeletal muscles are comprised of a mixture of fibers categorized as slow (type I) or fast (type II) based on their morphologic, metabolic, and mechanical properties. Slow muscle fibers are predominantly dependent on oxidative phosphorylation for energy, have a relatively high capillary density, are highly resistant to fatigue, contain type I myosin heavy chain (MHC), are small in size, have relatively slow isometric twitch properties, and exhibit a relatively slow shortening velocity^{1,2}. In contrast, fast fibers generally exhibit opposite characteristics, i.e., have higher dependence on the glycolytic pathways for energy, are less resistant to fatigue, have relatively fast isometric twitch properties, and exhibit a faster shortening velocity^{1,2}. In general, the muscle speed properties reflect its fiber type composition³.

It is well documented that after a spinal cord injury there is marked atrophy and a pronounced shift in the MHC profile from type I to type II in the muscles that are innervated below the level of injury⁴⁻⁸. A reduction in the oxidative potential and an increase in the fatigability of hindlimb muscles comprised predominantly of fast fibers also have been reported in *in situ* preparations^{9,10}. Similarly, paralyzed human subjects show increased neuromuscular fatigability in muscles below the site of spinal cord injury in both acute and chronic^{11,12} stages after injury. Therefore, reversing or reducing this fatigability most likely would result in significant functional benefits for spinal cord injured subjects.

Nuclear hormone receptors have been shown to alter muscle fiber phenotype, oxidative capacity, and substrate usage^{13,14}. For example, peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors expressed in a variety of tissues and play key roles in lipid metabolism¹⁵. One PPAR subtype, PPAR δ plays a critical role in transcriptional regulation of skeletal muscle metabolism by switching fuel usage from glucose and fatty acids to predominantly fatty acids, thereby increasing the oxidative capacity of the muscle^{16,17}. Overexpression of constitutively active PPAR δ in skeletal muscles of transgenic mice increased the number of oxidative muscle fibers and enhanced running endurance by ~100% compared to wild-type littermates¹⁴. Similarly, *in vivo* transfection of active PPAR δ (VP16-PPAR δ) in pre-existing fibers of the extensor digitorum longus (EDL) muscle of adult rats resulted in a transformation of fast fibers towards a slower, more oxidative phenotype¹⁸. For example, the number of I/IIa hybrids tripled, IIa fibers increased from 14% to 25%, and IIb fibers decreased from 55% to 45% in PPAR δ -transfected EDL muscles after 14 days. These findings suggest that PPAR δ modulation could be used to prevent the increased fatigability and shift towards faster mechanical and metabolic properties that occur after muscle paralysis.

The purpose of this study was to determine and compare the effects of PPAR δ overexpression on the phenotype of the medial gastrocnemius (MG, a predominantly fast

plantar flexor) muscle in intact and complete spinal cord transected (ST) adult wild-type and PPAR δ transgenic mice. We hypothesized that overexpression of PPAR δ , which results in a higher percentage of slow (high oxidative and fatigue resistant) fibers than observed in wild-type mice^{18,14}, would attenuate the decrease in fatigue resistance normally observed in predominantly fast muscles after complete ST^{19,9,10}. The MG was studied because it is a mixed fast muscle and has a fiber type composition and distribution that is representative of most muscles in the hindlimb. We find that overexpression of PPAR δ enhances fatigue resistance of the MG, and this enhancement is maintained after ST, suggesting that activation of its target gene network could have potential therapeutic benefits.

MATERIALS AND METHODS

Experimental groups

Age- and gender-matched mice used in the study were derived from the strain originally described by Wang, et.al.¹⁴ Adult PPAR δ transgenic and age-matched wild-type (WT) littermates (n=6–8/group) were assigned randomly to a WT-Control (Con), WT-ST, PPAR δ -Con, and PPAR δ -ST group. All mice in the ST groups underwent ST surgery. All procedures were approved by the UCLA Office of Animal Research Oversight and followed the American Physiological Society Animal Care Guidelines.

Surgical procedures and animal care

All surgical procedures were performed under aseptic conditions as described previously²⁰. All ST mice were anesthetized deeply using isoflurane gas via facemask to effect. A longitudinal midline skin incision was made dorsal to the spinal column from the T₆–T₁₀ levels. The muscles overlying the spinal column were separated from the spinal column, and a partial laminectomy was performed to expose the spinal cord at the T₇–T₈ levels. The spinal cord, including the dura, then was transected completely with micro-dissection scissors. To verify the completeness of the transection, 1) a probe was passed between the cut ends of the spinal cord, and 2) the cut ends of the spinal cord were lifted gently using fine forceps. Gelfoam was packed between the cut ends of the spinal cord. The fascia surrounding the spinal column was sutured (5.0 Dexon) over the transection site, and the skin incision closed using 5.0 Ethilon. The mice were placed in an incubator until fully awake and transferred to individual plastic cages amply lined with CareFresh. Baytril, a general antibiotic, and Buprenex, an analgesic, were administered (5 mg/kg and 0.05 mg/kg subcutaneously twice daily, respectively) during the first 2 days of recovery. Post-surgical care included manual expression of the bladder three times daily for the first week and twice daily thereafter as previously described^{21,20}. Once daily the hindlimbs of each mouse were stretched gently through a full range of motion at each joint to help sustain joint mobility.

In situ mechanical properties

Four weeks after ST, the MG from all mice were tested for their *in situ* mechanical properties as described by Roy et al.²². The mice were anesthetized with a mixture of ketamine (133 mg/kg body weight) and xylazine (9 mg/kg body weight) and given supplemental doses of ~20% the initial dose as needed. The MG nerve was isolated carefully with a glass probe, cut as far proximal as possible, and placed on a bipolar silver electrode

for stimulation. The MG tendon was freed at its distal end and secured to a stiff wire near the musculotendinous junction that was attached to a force transducer-lever system (Cambridge Instruments) to measure the muscle isometric properties (see below). The mice were placed in a prone position on a heating pad maintained at $36 \pm 1^\circ\text{C}$. Using a combination of bars, pins, and clamps, the hip, knee, and ankle joints were stabilized at $\sim 90^\circ$ with the MG in a horizontal position. The skin in the lower leg was freed from connective tissue and used to form a warm mineral oil pool that was maintained at $35 \pm 1^\circ\text{C}$ using radiant heat. The temperature of the muscle was allowed to equilibrate with the mineral oil for at least 30 min prior to physiological testing, and the temperature was monitored with a digital thermocouple inserted in an adjacent muscle with feedback capability.

The average time-to-peak-tension (TPT), half-relaxation time (HRT), and maximum twitch tension (Pt) were determined from 10 consecutive isometric twitch responses at the optimum muscle length at 2 times threshold stimulation intensity. Peak isometric tension development was measured at frequencies of 5, 10, 20, 30, 40, 50, 75, 100, 200, and 250 Hz for a duration (between 100 ms and 1 s) that resulted in a plateau in the tension output. The highest tension achieved regardless of stimulation frequency was considered the maximum isometric tetanic tension (P_o). The isometric fatigue properties were determined by 330-ms trains of 40 Hz delivered once per sec for 2 min, and the fatigue index was calculated as a ratio of the tension produced at 2 min divided by the maximum tension measured during the fatigue test. Specific tension was calculated as $P_o/\text{muscle weight (g/mg)}$.

Tissue harvesting

The MG muscle was removed bilaterally, trimmed of fat and connective tissue, and weighed (wet weight). The MG muscles used for the physiology experiments were pinned on cork at near physiological length, quick-frozen in isopentane cooled with liquid nitrogen, and stored at -80°C until they underwent histochemical and immunohistochemical analyses. The muscles not used for the physiological experiments were quick frozen in liquid nitrogen and stored at -80°C until they underwent RNA analyses.

Quantitative RT-PCR

Assays were performed as described previously²³. Briefly, for each biological sample, quantitative PCR reactions were performed in triplicate, and expression of PPAR δ was normalized to *Gapdh* expression. The averaged values of PPAR δ in the MG were expressed relative to the WT values.

Quantitative RT-PCR

To determine if expression of PPAR δ is altered after ST, we analyzed PPAR δ expression by quantitative RT-PCR (QPCR) in VP16-PPAR δ transgenic mice and WT controls as described previously²³. Briefly, for each biological sample, quantitative PCR reactions were performed in triplicate, and expression of PPAR δ was normalized to *Gapdh* expression. The results confirmed that transgenic mice had an 18- to 20-fold increase in expression of PPAR δ compared to WT-Con (data not shown).

Immunohistochemical analyses

Serial cross sections (10 μm thick) from the mid-belly of each MG were cut in a cryostat maintained at -20°C and mounted on gelatin-coated slides. The MHC profiles of ~ 100 adjacent fibers from a representative area from deep (the region of the muscle near the tibia) and superficial (the region of the muscle away from the tibia) regions were determined. A series of monoclonal antibodies specific to mouse MHC isoforms were used for the immunohistochemical analyses as described by Talmadge et al.⁸. Anti-slow and anti-fast antibodies (Vector Laboratories, Burlingame, CA) were used to identify slow and fast MHC isoforms, respectively. The avidin-biotin immunohistochemical procedure was used to localize and amplify the antigen-antibody binding complex (Vectastain ABC kits, Vector Laboratories). Additional tissue sections were stained without primary antibody incubation to control for nonspecific binding. Fibers were considered to contain a specific MHC if there was a visually detectable reaction in the fiber to the appropriate monoclonal antibody. Fibers were classified as type I (positive for the slow antibody), type I+II (positive for both the slow and fast antibodies), and type II (positive for the fast antibody).

Succinate dehydrogenase (SDH) analysis

Histochemical staining for succinate dehydrogenase (SDH) was carried out as described by Luquet et al.²⁴. Briefly, fresh-frozen sections from the mid-belly of the MG were cut at 10 μm in a cryostat and placed on gelatin-coated slides. Slides then were incubated in 0.5 mM phosphate buffer (pH 7.6) containing sodium succinate and nitro-blue tetrazolium for 20 min at 37°C . Slides were transferred to 0.9% NaCl briefly, dehydrated through a serial dilution of ethanol, cleared with Citri-solv (Fisher Scientific, Pittsburgh, PA), and mounted with Permount. Individual fibers were assessed qualitatively as staining either darkly or lightly for SDH. To assess the level of SDH staining across the entire muscle cross section, the area containing a relatively high percentage of fibers staining darkly for SDH was measured using Axiovision (Zeiss), divided by the total area of the muscle cross section, and reported as a percent.

Statistical analyses

All data are presented as mean \pm SEM. Overall mean differences were determined using a 2×2 analysis of variance (ANOVA) model followed by the Neuman-Kuels *post hoc* test for determining pairwise mean differences. The Prism software package (GraphPad) was used for all analyses. Significant differences were determined at $P < 0.05$.

RESULTS

Body and MG weights

The initial mean body weights were similar across groups: WT-Con, WT-ST, PPAR δ -Con, and PPAR δ -ST were 23.3 ± 0.8 , 23.9 ± 0.7 , 22.9 ± 0.5 , and 23.5 ± 0.3 g, respectively. At the 4-week time point, however, the mean body weights for the WT-ST and PPAR δ -ST groups were 13% and 18% lower than their respective Con groups (Fig. 1A), consistent with previous reports^{25,26}.

At the 4-week time point, the mean absolute MG weight was 26% smaller in the PPAR δ -Con compared to the WT-Con group (Fig. 1B). The absolute MG weights were 24% and 37% smaller in the WT-ST and PPAR δ -ST mice compared to their respective Con groups. In addition, the absolute MG weight was 38% smaller in the PPAR δ -ST compared to the WT-ST group. Relative (to body weight) MG weight was 35% smaller in the PPAR δ -Con than in the WT-Con group (Fig. 1C). The relative weights of the WT-ST and PPAR δ -ST groups were 14% and 16% smaller than their respective Con groups, with the relative weight of the PPAR δ -ST group being 26% smaller than the WT-ST group. These results indicate that the effects of ST on all measures of muscle weight were greater in the PPAR δ than in the WT mice.

MG mechanical properties

Mean twitch TPT was longer in the PPAR δ -Con than in the WT-Con group and longer in the PPAR δ -ST than in the WT-ST group (Fig. 2A). ST resulted in a shorter TPT in the WT-ST than WT-Con group, but there was no effect in the PPAR δ mice. Mean HRT was longer in both PPAR δ groups compared to their respective control groups (Fig. 2B). Unexpectedly, mean HRT was longer in the PPAR δ -ST than the PPAR δ -Con group. Overall these results are consistent with a lengthening of the twitch duration of the MG with PPAR δ overexpression.

The mean absolute tension produced in the PPAR δ -Con was lower than in the WT-Con group at all stimulation frequencies above 150 Hz (Fig. 3A). These values were lower than both Con groups at all frequencies above 50 Hz and above 40 Hz for the WT-ST and PPAR δ -ST groups, respectively. In addition, the mean absolute tension was lower in the PPAR δ -ST than the WT-ST at all frequencies above 40 Hz. Interestingly, the mean relative tension (expressed as a percent of P_o) was similar for the PPAR δ -Con, WT-Con, and WT-ST groups at all stimulation frequencies (Fig. 3B). In contrast, the tensions were higher (curve shifted to the left) in the PPAR δ -ST than all other groups at all frequencies up to 50 Hz, and higher than WT-ST up to 75 Hz. This leftward shift in the curve in the PPAR δ -ST group suggests a shift toward slower muscle properties.

The mean P_t was quite variable and was not different across groups (Fig. 4A). The mean P_o , however, was 16% lower in the PPAR δ -Con than in the WT-Con group (Fig. 4B), and ST resulted in a mean P_o that was 35% lower in the WT-ST than the WT-Con and 51% lower in the PPAR δ -ST than the PPAR δ -Con groups (Fig. 4B). Significantly, the mean P_o was 51% lower in the PPAR δ -ST than the WT-ST group. Mean P_t/P_o in the PPAR δ -ST was 70% and 32% larger than in the PPAR δ -Con and WT-ST groups, respectively (Fig. 4C). Specific tension was 22% lower in the PPAR δ -ST than in the PPAR δ -Con group (Fig. 4D). Finally, the fatigue index was 50% higher in the PPAR δ -Con than the WT-Con group and unexpectedly 70% higher in the PPAR δ -ST than in the WT-ST group, indicating a marked improvement in fatigue resistance with PPAR δ overexpression (Fig. 4E).

Fiber type composition is not affected in the MG of VP16-PPAR δ mice

The deep region of the MG of WT-Con mice was composed of 12, 1, and 87% types I, I+II, and II fibers, respectively (Fig. 5A). The MG superficial region in all groups was composed

of fibers containing only the type II isoform (Fig. 5B). There were no significant differences in the fiber type composition in either the deep or superficial regions of the MG among groups.

Oxidative profile of the MG

The area of the MG cross-section with a high proportion of oxidative fibers was ~40% greater in both PPAR δ groups compared to their respective control group (Fig. 6). In addition, this percent was lower in the PPAR δ -ST than the PPAR δ -Con group.

DISCUSSION

There is growing evidence that PPAR δ has a strong influence in the maintenance of and transformation to the slow and oxidative skeletal muscle phenotype. For example, a recent study of biopsies from the vastus lateralis muscles of elite athletes, spinal cord injured individuals, and normally active individuals showed a strong correlation between the amount of type I fibers and PPAR δ expression²⁷. Elite cyclists had the highest proportion of type I fibers and the highest expression of PPAR δ mRNA, whereas the spinal cord injured subjects had the lowest proportion of type I fibers (large increase in type IIb) and lowest PPAR δ expression levels. Accordingly, we explored whether enhanced PPAR δ expression in skeletal muscle could mitigate the shift towards faster mechanical properties, lower oxidative capacity, and increased fatigability associated with ST. Using the MG, an ankle plantar flexor comprised predominantly of fast fibers, our data clearly demonstrate that activation of the PPAR δ signaling pathway results in a shift towards slower isometric twitch and frequency-tension properties and reduced fatigability compared to WT-Con and that these properties are maintained for at least 4 weeks after a complete mid-thoracic ST.

Effects of PPAR δ overexpression on muscle weight

The effects of PPAR δ overexpression on muscle properties appear to be dependent on the type of muscle, i.e., slow vs. fast, and the mechanism by which the gene is expressed, i.e., transgenic vs. viral. For example, our studies show that in the soleus, a predominantly slow ankle plantar flexor, the relative muscle weight (compared to body weight) was 58% higher (data not shown), whereas in the MG it was 26% smaller in the PPAR δ transgenic compared to the WT littermates (Fig. 1). Similarly, although Lunde and colleagues¹⁸ did not report muscle weight data, consistent with this decrease in muscle mass of a fast ankle extensor is the report of an overall 14% decrease in fiber cross-sectional area in adult rat extensor digitorum longus muscles (a fast flexor) 5 days after they were transfected with a VP16-PPAR δ fusion construct. In contrast, in homozygous transgenic PPAR δ mice there were no significant changes in the absolute muscle weight in the tibialis anterior, soleus, and plantaris muscles compared to control littermates²⁴. The observed differences between the studies above could stem from developmental differences in PPAR δ transgenic expression, i.e., whether the PPAR δ is overexpressed using a CRE-recombinase system²⁴ or through generation of a VP16-PPAR δ construct¹⁴, or due to differences in the promoters, such as VP16 used here. Moreover, the lack of change in body weight despite the marked decrease in muscle weight may reflect increases in bone density as previously observed in ovariectomized mice given the PPAR β/δ -specific agonist GW501516²⁸.

Effects of PPAR δ overexpression on skeletal muscle isometric mechanical properties

The atrophy and associated decrease in maximum force generation in the MG of both WT and PPAR δ mice in response to ST is consistent with that observed in a number of species and spinal cord injury models^{22,10}. The shorter TPT in WT-ST compared with WT-Con also is consistent with faster isometric twitch speed properties after ST as reported previously^{29,9,10}. In contrast, there was no difference in TPT in the PPAR δ -Con and PPAR δ -ST and a longer HRT after ST. This prolonged HRT is consistent with the shift of the frequency-tension curve to the left in the PPAR δ transgenic mice, indicating that the MG has slower isometric mechanical properties after ST. These results may reflect an adaptation in calcium kinetics during excitation-contraction coupling, particularly calcium re-uptake during relaxation^{30,31} and are consistent with the reported increase in slow and decrease in fast forms of troponin I in PPAR δ transgenic mice¹⁴. In addition, changes in the function and concentration of Ca²⁺-ATPase have been suggested to explain, at least in part, the slowing of relaxation time in the quadriceps muscles of spinal cord injured subjects 24 weeks post-injury³².

Effects of PPAR δ overexpression on skeletal muscle MHC composition

The MHC composition between the deep and superficial regions was not different between the WT and PPAR δ groups. This is in contrast to what was observed in normal adult rat EDL muscles transfected with a constitutively active PPAR δ construct for 14 days in which there was a shift in the MHC fiber type composition towards the slower phenotype, i.e., increased type I+IIa and IIa fibers with concomitant decreases in MHC IIb¹⁸. The number of “hybrid” I+II fibers was not different in the PPAR δ -Con compared to the WT-Con group in our study. Even 4-weeks after ST the number of hybrid fibers in both the deep and superficial regions did not change. Thus in our hands it appears that the appearance of hybrid fibers often associated with changes in muscle activity did not occur as reported previously in the soleus muscle after 360 days of ST in adult rats³³. The mice used in our study are born with excess PPAR δ , and any changes associated with increased PPAR δ may have occurred during development. The conversion of type II to type I fibers has been observed in mice that overexpress calcium/calmodulin-dependent protein kinase IV or PGC-1 α ^{34,35}, but overexpression of PPAR δ does not promote the appearance of type I fibers in TA and plantaris muscles²⁴. This is consistent with our observations that show no changes in MHC composition of type I fibers across groups. We did not distinguish between the different fast MHC isoforms, i.e., IIa, IIx, and IIb, thus we cannot determine if there were any changes in the expression of these isoforms among groups.

Effects of PPAR δ overexpression on skeletal muscle metabolic and fatigue properties

Lunde et al.¹⁸ reported that the overall fiber SDH activity level in the EDL based on quantitative histochemistry was 43% higher in the PPAR δ -transfected than control rats. SDH activity was elevated in all fast fiber types. Type IIb fibers, that normally have the lowest levels of SDH activity, had levels similar to that normally found in IIx fibers, and type IIx and IIa fibers had 82% and 20% increases in SDH activity, respectively. Luquet et al.²⁴ also reported an increase in the number of fibers staining darkly for SDH in the tibialis anterior and plantaris muscles and in the level of citrate synthase activity in the tibialis

anterior of double transgenic PPAR δ mice. In our study we observed an increase in the percent of the muscle cross section containing a high proportion of fibers staining darkly for SDH, a finding consistent with the overall increase in oxidative capacity reported with PPAR δ overexpression previously^{18,24,36,14}. Wang et al.¹⁴ found an approximately 2-fold increase in type I fibers (using a metachromic dye-ATPase method) in the gastrocnemius of PPAR δ transgenic mice and reported 67% and 92% increases in running time and distance, respectively, when the mice were run to exhaustion on an oxygen-infused enclosed treadmill. In the present study, the fatigue resistance of the MG was significantly higher in the PPAR δ -Con than in the WT-Con group, consistent with the reported higher oxidative capacity and the higher number of fatigue-resistant fibers in PPAR δ transgenic mice^{18,14}.

PPAR δ maintains skeletal muscle fatigue properties after ST

The MG in PPAR δ transgenic mice was more fatigue resistant than in WT mice, and this difference (70%) was maintained after ST. This is important because a decrease in fatigue resistance is one of the primary deficits observed after spinal cord injury in animal models and in humans³⁷. Most studies of spinal cord injury report a shift in the MHC composition from slow type I and fast type IIa towards fast type IIx and IIb that may contribute to greater fatigability due to the lesser efficiency of fast vs. slow myosin³⁸. This shift in fiber type composition also is associated with a decrease in the oxidative potential of the muscle that directly and negatively impacts its fatigue resistance properties. Our results demonstrate that PPAR δ overexpression can change the isometric mechanical properties of a predominantly fast, fatigable muscle towards a more oxidative, fatigue resistant muscle. Moreover, these properties are maintained even 4 weeks after complete ST. These results are particularly important when considering the advances that have been made in rehabilitating motor function in spinal cord injured subjects using a number of interventions such as motor training, spinal cord epidural stimulation, and/or administration of pharmacological agents³⁹⁻⁴¹. The maintenance of fatigue resistance in the muscles affected by spinal cord injury certainly would be beneficial for the level of recovery. Moreover, attenuating this response could have important therapeutic implications given the deleterious effects that increased fatigability has on muscle function and subsequent locomotor performance after ST.

Limitations

How the changes in the contractile properties are linked to changes in the MHC profile (composition) and other muscle-related properties, i.e., SERCA, troponin, etc remain unknown and should be considered to further elucidate the mechanisms (pathways) by which PPAR δ may be targeting muscle function after a ST. Although the myosin isoform is linked closely to contractile speed³, it is clear that it is not the only controller. This is readily obvious when considering the large range in maximum shortening velocity for a population of single muscle fibers expressing the same MHC isoform⁴². Our data also show that the metabolic changes as reflected by SDH staining intensity do not correlate with changes in MHC composition, suggesting that the oxidative potential of fibers in a muscle are not strongly dependent on muscle fiber phenotype. Since we were unable to separate the type II MHC isoforms, however, a more detailed analysis of the MHC composition may show that there is an association between the metabolic changes and the subtypes of fast MHC

isoforms in the MG, an extensor muscle comprised predominantly of fast fibers. Whether the adaptations induced by PPAR δ administration would be similar in a predominantly slow muscle such as the soleus is unknown.

Therapeutic implications

Muscle phenotype changes are likely to be influenced by a cascade of highly coordinated events including, but not limited to, those controlling myosin protein composition, metabolism, mitochondrial function, and vascularization. Factors such as diet, gene overexpression, and pharmacological interventions can trigger these events, but these factors most likely will simultaneously impact other physiological such as cardiovascular, respiratory, and other support systems. Overexpression of the nuclear hormone receptor estrogen receptor-related receptor γ (ERR γ) enhances the type I muscle phenotype in the normally fast gastrocnemius muscle and increases its vascularization and mitochondria content resulting in an increased resistance to fatigue¹³. Furthermore mice with ERR γ -remodeled muscle have an increase in oxygen consumption and lower respiratory exchange ratio and a subsequent increase in endurance and resistance to weight gain. Similarly, administration of the PPAR δ agonist GW501516 results in a robust activation of genes involved in lipid metabolism such as uncoupling protein 3 (Ucp3), muscle carnitine palmitoyl transferase I (mCPT I), and pyruvate dehydrogenase kinase 4 (Pdk4)^{36,43}. PPAR δ agonism however, does not activate mitochondrial gene expression and function *in vitro* and *in vivo* nor does it induce a fiber type switch from glycolytic to oxidative⁴³. In fact the effects of PPAR δ agonism increases mitochondrial gene expression only when combined with exercise³⁶, demonstrating that pharmacological intervention alone is not sufficient to have a beneficial effect on factors such as running time and distance, elements that are critical when developing a potential rehabilitative paradigm. Furthermore, PPAR δ agonism induced by GW610742X administration appears to have a positive therapeutic potential for type II diabetic patients. The potential has limited or negative long-term implications, as PPAR δ agonism can impair muscle function by inhibiting carbohydrate oxidation during exercise at intensities where carbohydrates are the mainstay of energy delivery⁴⁴. Thus, the significant differences in metabolic and biochemical properties in skeletal muscles of PPAR δ agonism vs. PPAR δ overexpression suggest the complex nature of this gene and its potential as a therapeutic intervention, and warrants further investigation.

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ABBREVIATIONS

Con control

CSA	cross-sectional area
EDL	extensor digitorum longus
ERRγ	estrogen receptor-related receptor γ
HRT	half-relaxation time
MG	medial gastrocnemius
MHC	myosin heavy chain
PDK4	pyruvate dehydrogenase kinase 4
PPARδ	peroxisome proliferator-activated receptor δ
Pt	maximum twitch tension
Po	maximum tetanic tension
SDH	succinate dehydrogenase
ST	spinal cord transection
TPT	time-to-peak tension
WT	wild-type

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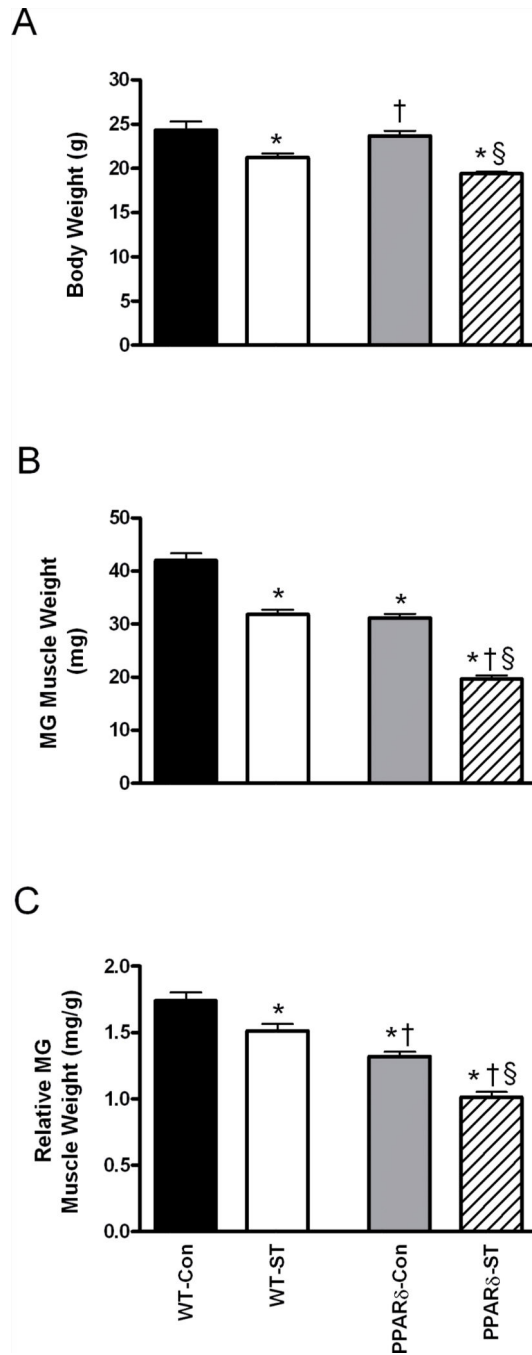


Figure 1. Body weight (A) for wild-type Control (WT-Con, n=8), WT-spinal cord transected (WT-ST, n=7), PPAR δ -Con (n=6), and PPAR δ -ST (n=8) groups. Absolute (B) and relative to body weight (C) medial gastrocnemius (MG) weights for each group. Values are means \pm SEM. *, †, and § denote a significant difference from WT-Con, WT-ST, and PPAR δ -Con groups, respectively, at $P < 0.05$.

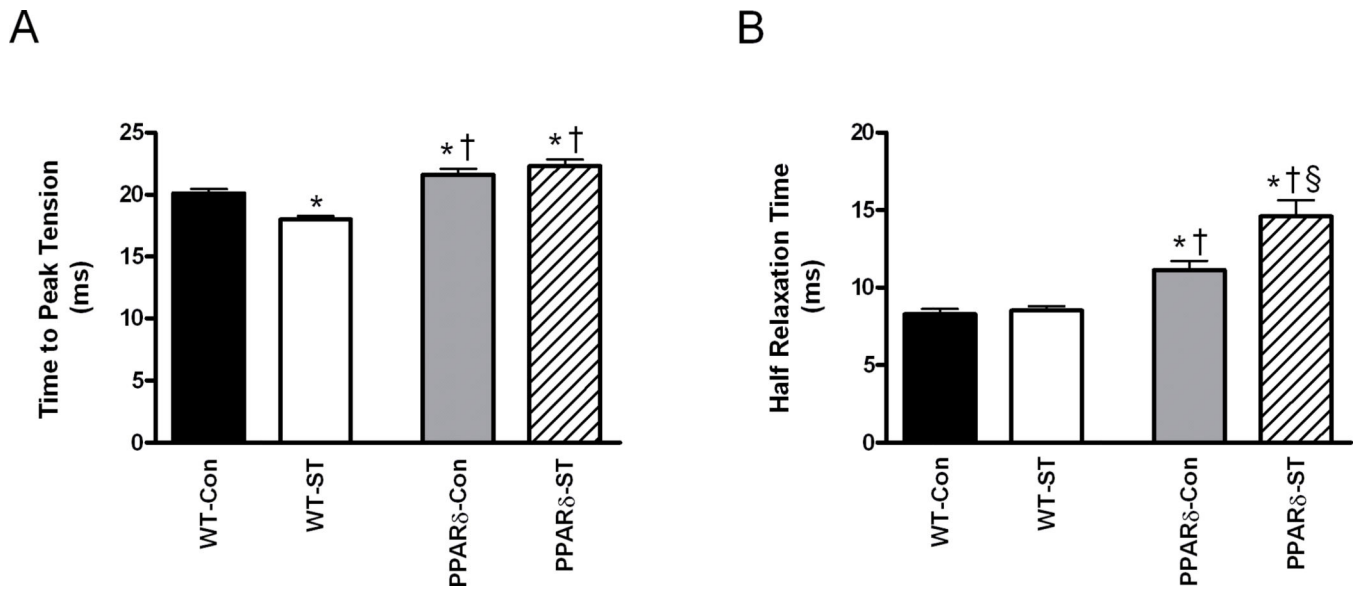


Figure 2.

Time-to-peak-tension (A) and half-relaxation times (B) of the MG from WT-Con, WT-ST, PPAR δ -Con, and PPAR δ -ST groups at the end of the 4-week experimental period. Values are means \pm SEM. *, †, and § denote a significant difference from WT-Con, WT-ST, and PPAR δ -Con groups, respectively, at $P < 0.05$.

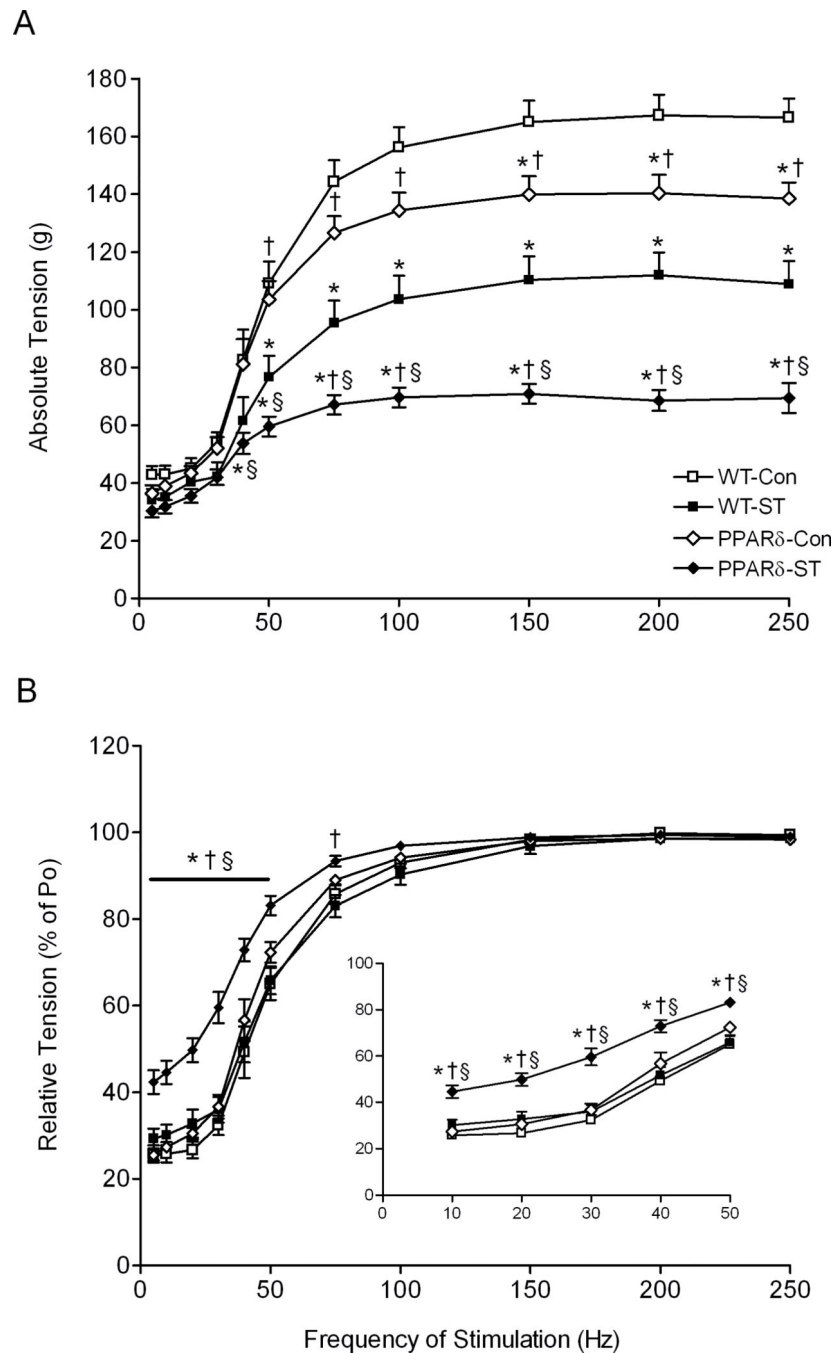


Figure 3. Absolute (A) and relative [% of maximum tetanic tension (B)] frequency of stimulation-tension responses for the MG muscles for the WT-Con, WT-ST, PPAR δ -Con, and PPAR δ -ST groups at the end of the 4-week experimental period. Inset in (B) is the expanded relative frequency of stimulation-tension response from 10 to 50 Hz. Values are shown as means \pm SEM. P_o , maximum tetanic tension. *, †, and § denote a significant difference from WT-Con, WT-ST, and PPAR δ -Con groups, respectively, at $P < 0.05$.

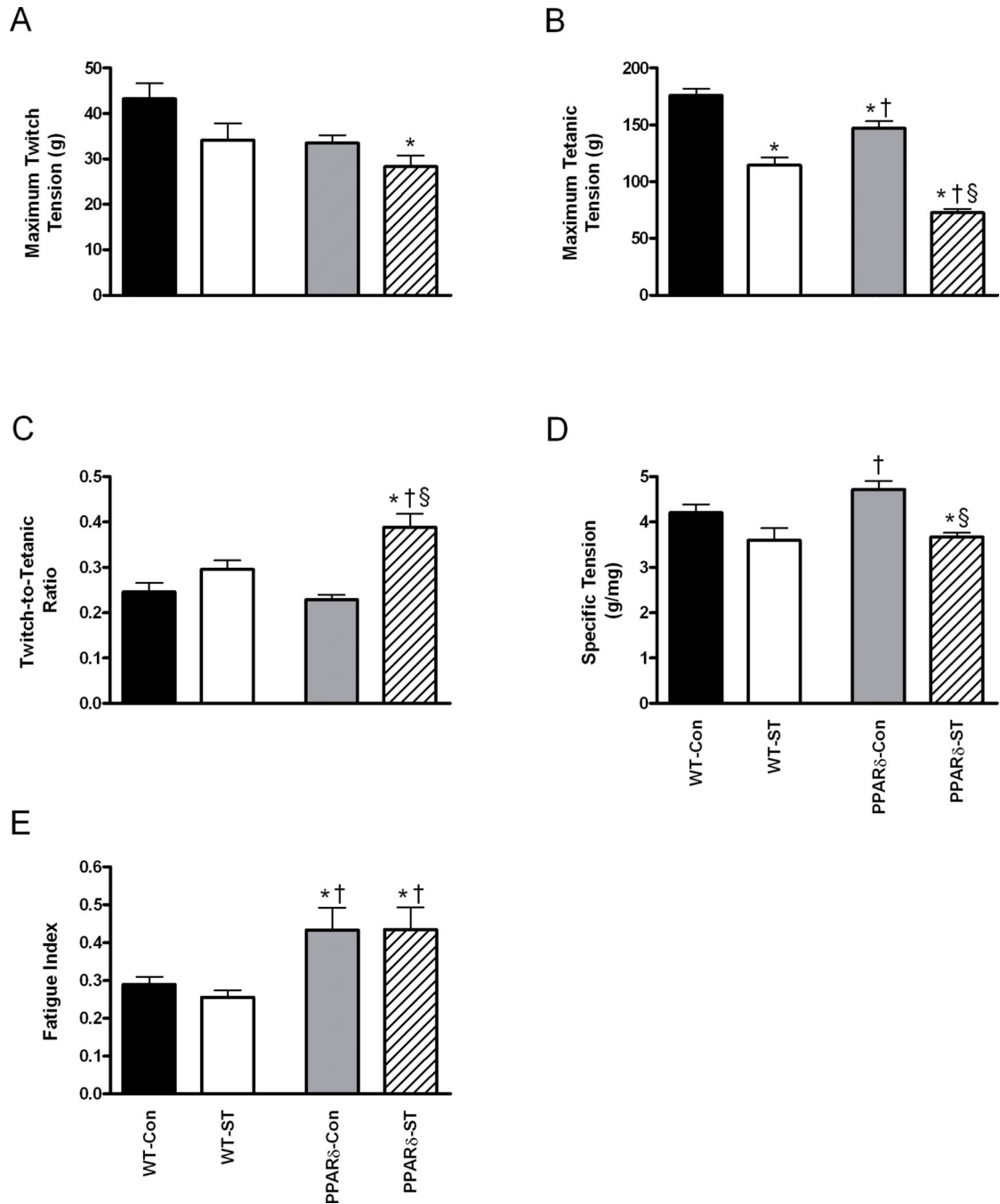


Figure 4. Maximum twitch tension (A), maximum tetanic tension (B), twitch-to-tetanic tension ratio (C), specific tension (D), and fatigue index (E) of the MG from WT-Con, WT-ST, PPAR δ -Con, PPAR δ -ST groups at the end of the 4-week experimental period. Specific tension is expressed as maximum tetanic tension (g) per muscle weight (mg). Values are means \pm SEM. *, †, and § denote a significant difference from WT-Con, WT-ST, and PPAR δ -Con groups, respectively, at $P < 0.05$.

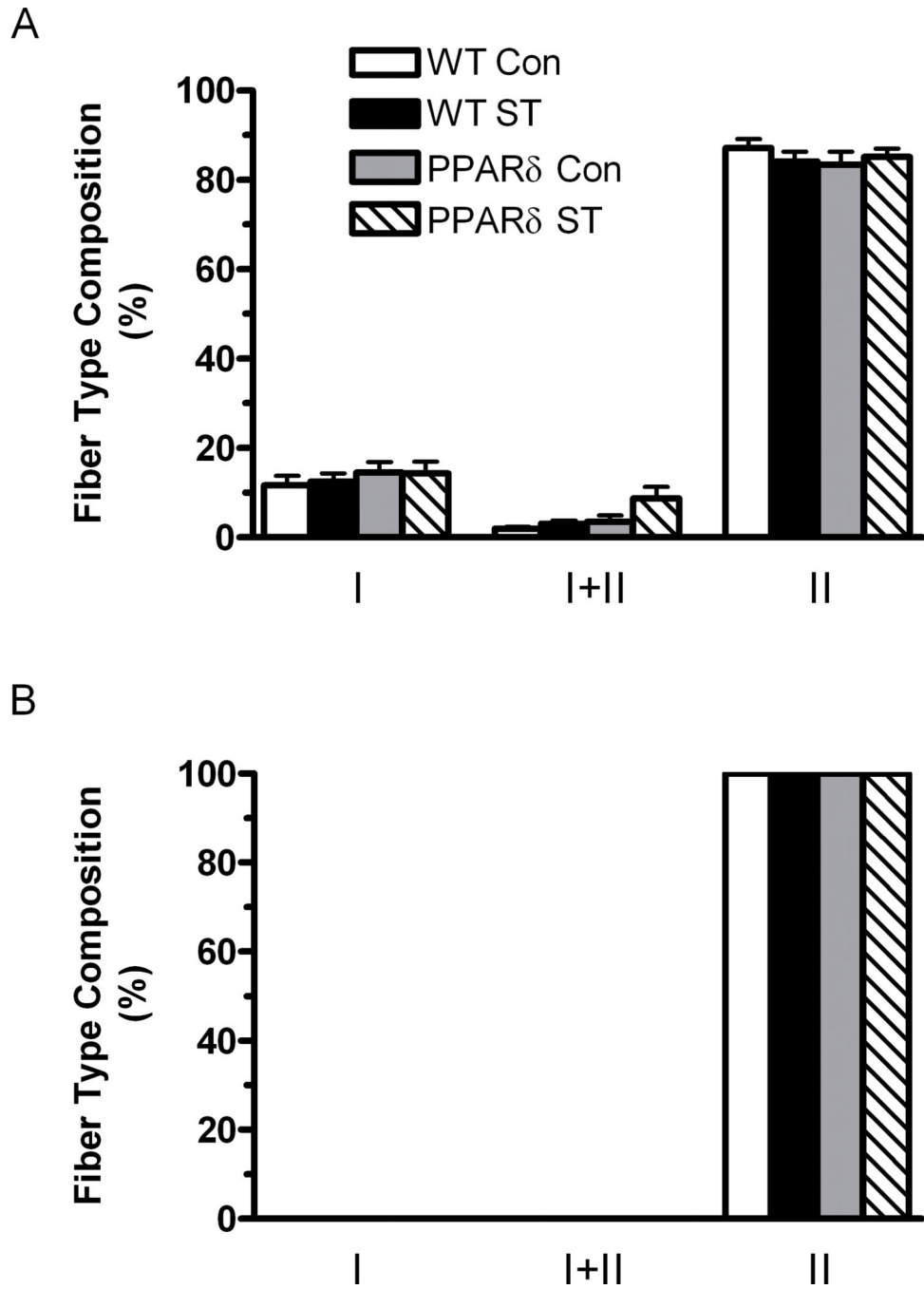


Figure 5. Fiber type composition determined by MHC immunohistochemistry. The data are expressed as a percentage of the total number of fibers sampled in the deep (A) (close to the bone) and superficial (B) (away from the bone) regions of the MG from WT-Con, WT-ST, PPAR δ -Con, and PPAR δ -ST groups. Values are means \pm SEM.

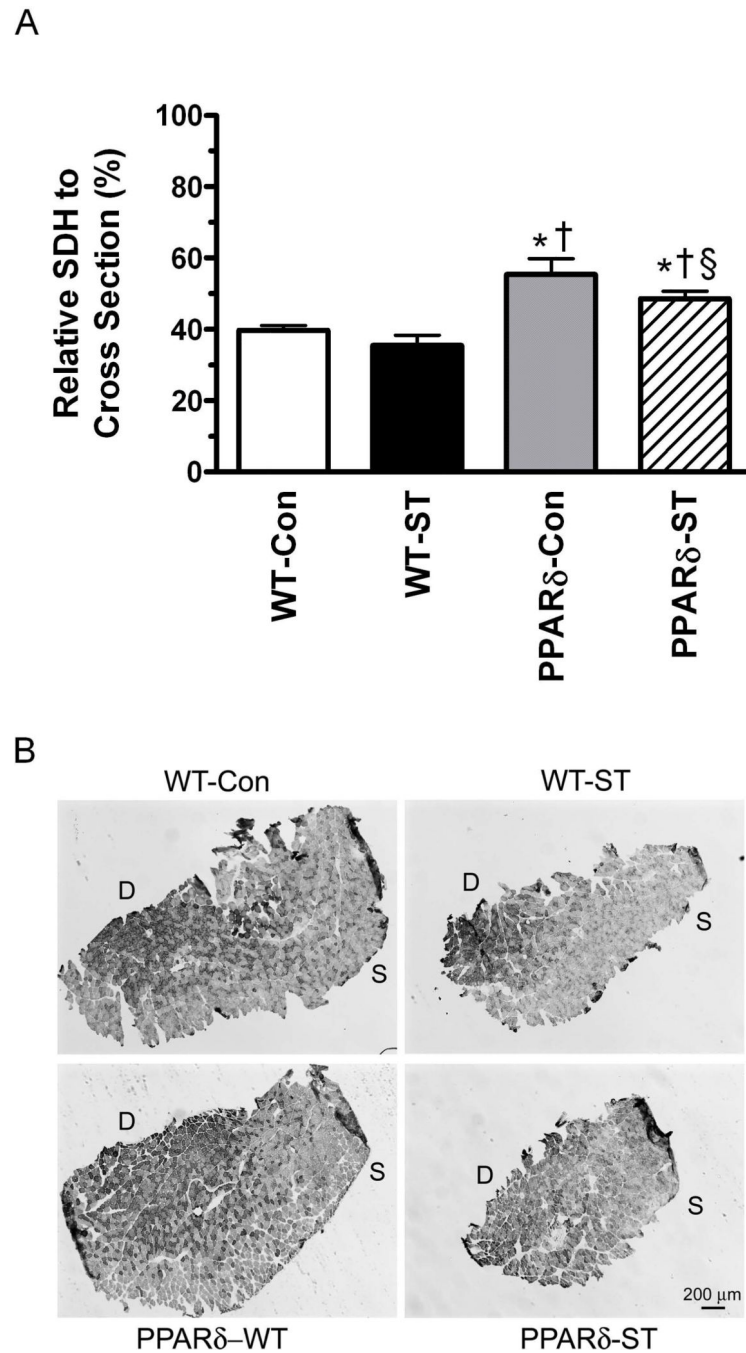


Figure 6. Relative SDH levels per whole muscle cross section (A) and representative cross-sections from the mid-belly of the MG (B) from WT-Con, WT-ST, PPAR δ -Con, and PPAR δ -ST groups. Values are means \pm SEM. D, deep region of the muscle close to the bone; S, superficial region of the muscle away from the bone. *, †, and § denote a significant difference from WT-Con, WT-ST, and PPAR δ -Con groups, respectively, at $P < 0.05$.