Metallothionein deficiency leads to soleus muscle contractile dysfunction following acute spinal cord injury in mice

Lara R. DeRuisseau,1 Daniel M. Recca,2 Jacqueline A. Mogle,3 Michelle Zoccolillo,1 and Keith C. DeRuisseau2

1Department of Biology, Le Moyne College, and Departments of 3Exercise Science and 3Psychology, Syracuse University, Syracuse, New York

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Metallothionein deficiency leads to soleus muscle contractile dysfunction following acute spinal cord injury in mice. Am J Physiol Regul Integr Comp Physiol 297: R1795–R1802, 2009. First published October 14, 2009; doi:10.1152/ajpregu.00263.2009.—Metallothionein (MT) is a small molecular weight metal binding protein possessing metal binding and free radical scavenging properties. We hypothesized that MT-1/MT-2 null (MT−/−) mice would display exacerbated soleus muscle atrophy, oxidative injury, and contractile dysfunction compared with the response of wild-type (WT) mice following acute spinal cord transection (SCT). Four groups of mice were studied: WT laminectomy, WT transection, MT−/− laminectomy (MT−/− lami), and MT−/− transection (MT−/− trans). Laminectomy animals served as surgical controls. Mice in SCT groups experienced similar percent body mass (BM) losses at 7 days postinjury. Soleus muscle mass (MM) and MM-to-BM ratio were lower at 7 days postinjury in SCT vs. laminectomy mice, with no differences observed between strains. However, soleus muscles from MT−/− trans mice showed reduced maximal specific tension compared with MT−/− lami animals. Mean cross-sectional area (μm²) of type I and type IIA fibers decreased similarly in SCT groups compared with laminectomy controls, and no difference in fiber distribution was observed. Lipid peroxidation (4-hydroxynonenal) was greater in MT−/− transex vs. MT−/− lami mice, but protein oxidation (protein carbonyls) was not altered by MT deficiency or SCT. Expression of key antioxidant proteins (catalase, manganese, and copper-zinc superoxide dismutase) was similar between the groups. In summary, MT deficiency did not impact soleus MM loss, but resulted in contractile dysfunction and increased lipid peroxidation following acute SCT. These findings suggest a role of MT in mediating protective adaptations in skeletal muscle following disuse mediated by spinal cord injury.

metallosome; oxidative stress; spinal cord transection; antioxidant; stress protein

Metallothionein (MT) is a small molecular weight metal binding protein expressed as four isoforms in humans, mice, and other mammals (reviewed in Ref. 18). Metallothionein-1 (MT-1) and metallothionein-2 (MT-2) proteins are ubiquitously expressed and are inducible isoforms of MT. Conversely, metallothionein-3 (MT-3) and metallothionein-4 (MT-4) display more limited expression and are found primarily in the brain and skin, respectively. MT appears to be involved in numerous cellular processes that include free radical scavenging, intracellular zinc transport and storage, metal detoxification, and zinc exchange with metalloproteins (reviewed in Ref. 10). MT is an effective scavenger of both reactive oxygen and nitrogen species (5, 25, 36) that may stem, in part, from the numerous sulfhydryl groups associated with the protein (21, 39, 42, 47). Therefore, MT appears to serve as a key defense mechanism against the damaging effects of free radicals (2, 32).

In skeletal muscle, basal MT expression is low, but expression becomes largely upregulated in response to disuse (11, 24, 34, 44). Consistent with the response of other cell types, it is generally thought that skeletal muscle MT upregulation represents a protective adaptation, although no studies have examined the functional role of MT in skeletal muscle. To date, studies investigating skeletal muscle MT have been limited to reports of altered mRNA and protein expression levels (11, 19, 22, 24, 28, 31, 34, 35, 38, 45). For example, significant elevations in MT protein levels have been observed in the soleus following limb immobilization (24). Large elevations in MT mRNA have been observed in gastrocnemius and unloaded rat diaphragm muscles following denervation and mechanical ventilation, respectively (11, 34). Moreover, a recent study on spinal cord injured patients showed upregulation of vastus lateralis MT protein and gene transcripts in the days following spinal cord injury (44). Collectively, these data suggest that MT may play a role in mediating adaptations of skeletal muscle to disuse, but a direct test of this postulate has not been reported.

In the current experiment, we employed mice deficient in MT-1 and MT-2 proteins (i.e., MT−/−) to test the hypothesis that MT deficiency would augment atrophy and oxidative injury and lead to contractile dysfunction of the soleus muscle following disuse mediated by acute spinal cord injury. Spinal cord transection (SCT) was the technique employed to examine spinal cord injury-mediated muscle disuse, as numerous reports have documented the deterioration of muscle mass (MM) that occurs following this form of spinal cord injury (13, 23, 27, 37, 41). In short, the results indicate that soleus muscles obtained from spinal cord injured MT−/− animals demonstrated impaired maximal force generation per cross-sectional area (CSA) and increased lipid peroxidation, compared with muscles obtained from MT−/− surgical controls. These findings support the notion that MT may serve a protective role in skeletal muscle in response to stressful stimuli.

EXPERIMENTAL METHODS

Ethical approval and experimental animals. Animal use was approved by the Syracuse University Institutional Animal Care and Use Committee and followed guidelines established by the American Physiological Society. MT-1/MT-2 null (129S7/SvEvBrd-Mt1tm1Bri Mt2tm1Bri/J) male mice (33) and appropriate wild-type controls were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed two to four per cage until surgery at ~6 mo of age. Mice received food and water ad libitum and were maintained on a 12:12-h light-dark cycle. Four groups of mice were studied: wild-type laminectomy...
(WT lami, n = 7), wild-type transection (WT trans, n = 12), MT−/−
laminectomy (MT−/− lami, n = 5), and MT−/− transection (MT−/−
trans; n = 12).

Laminectomy and SCT at thoracic level T9. Animals were anesthe-
tized with intraperitoneal administration of ketamine (100 mg/ml) and
xylazine (20 mg/ml), and respiratory rate was continuously monitored
as a measure of anesthetic depth. The fur of the upper back was
shaved, and the skin was prepped with Betadine (Purdue Products L.P.,
Stamford, CT) and 70% alcohol. A midline incision was made with a
scalpel caudal to the ears ~2 cm in length. After reflecting the
skin and clearing fascia, the muscles on either side of the midline were
reflected. Using a surgical microscope, the T9 vertebrae were exposed.
The dorsal spinous processes and vertebrae were cleaned with a
microcurette, and a laminectomy was performed using microcon-
routers and/or spring scissors. Once bleeding was controlled with
gelfoam, an incision was made in the dorsal dura, and the spinal cord
was then exposed and cut using spring scissors at two places along the
T9 segment and removed. Complete transection was ensured when the
space between the two cut sections of spinal cord was suctioned using
a glass transfer pipette. In addition, care was taken to cut lateral spinal
nerve remnants. The severed ends of spinal cord typically retracted
~3 mm after this procedure. The musculature was sutured closed (4-0
silk) in layers, followed by closure of the skin (4-0 silk). The entire
procedure from anesthetic induction to skin suturing was ~1 h.
Buprenorphine was administered (0.5 mg/kg sc) immediately follow-
ing surgery, and mice were housed two to three animals per cage.
Animals in the laminectomy groups underwent the entire procedure,
except a SCT was not performed.

Postsurgical animal care. At least twice daily following SCT,
mouse bladders were expressed, and the size and taughtness of the
bladder was monitored. For the 7 days following SCT, sterile saline
was administered as needed (up to 2 ml at a time; subcutaneously)
along with the administration of a daily antibiotic (cefazolin 20 mg/kg
sc). Moist chow mixed with water, sucrose, and peanut butter was
placed on the cage bottom, along with several pieces of peanut butter
cereal mixed with Nutri-cal (Tomlyn, Buena, NJ) to maintain caloric
needs. Until all mice in the cage became active following surgery,
sc). Moist chow mixed with water, sucrose, and peanut butter was
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cereal mixed with Nutri-cal (Tomlyn, Buena, NJ) to maintain caloric
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needs. Until all mice in the cage became active following surgery.

Western blot assessment of antioxidant proteins and 4-hydroxynoneal
protein adducts. Soleus muscles were homogenized 1:10 in buffer [5
mM Tris-HCl, pH = 7.5; 1.0% Triton X-100; 0.1% SDS; 5 mM DTT; 1
mM PMSF (phenylmethanesulfonyl fluoride); 10 μM d-tubocurarine]
and placed on ice for 10 min (4°C). Protein content of the soluble fraction
was assessed by the method of Bradford (Sigma, St. Louis, MO). Proteins
were individually separated by SDS-PAGE via 12%
protein carbonyls.

Protein carbonyls. Protein carbonyl groups were measured as an
index of protein oxidation using the commercially available Oxyblot
assay kit (Millipore, Billerica, MA) in accordance with the manufac-
turer’s instructions. Briefly, 15 μg of soluble protein were reacted

output was recorded via a computerized data-acquisition system
(Dynamic Muscle Control v4.1.6; Aurora Scientific). After a 15-min
equilibration period, the muscle was stimulated using supramaximal
monophasic pulses of 0.5 ms to determine the optimum contractile
length by systematically adjusting the length of the muscle by using a
micrometer while evoking single twitches. Thereafter, all contractile
properties were measured isometrically at optimum contractile length.
To measure the force frequency response, each muscle was stimulated
supramaximally between 1 and 200 Hz (500-ms trains). Contractions
were separated by a 2-min recovery period. For comparative
purposes, soleus twitch and tetanic forces were normalized to the
muscle CSA (4).

Measurement of in vitro soleus muscle contractile function. The
soleus muscle was removed by the tendons and used for the assess-
mament of muscle contractile function. The right soleus was either stored for
biochemical analyses or embedded in optimum cutting temperature
compound or placed in a dissecting dish containing a Krebs-Hensley solution equilibrated
with a 95% O2-5% CO2 gas, where the proximal and distal tendons
were clipped with light-weight Plexiglas clamps (Harvard Apparatus,
Holliston, MA). The muscle was then suspended vertically between
two platinum stimulating electrodes in a jacketed tissue bath filled
with Krebs-Hensley buffer (37°C) containing 12 μM d-tubocurarine
and aerated with gas (95% O2-5% CO2; pH = 7.4). The distal end of
the muscle was attached to animmovable post, and the proximal end
was connected to a force transducer (301C; Aurora Scientific). Force
with 2,4-dinitrophenylhydrazone for 15 min. Following the reaction process, 10 μg of 2,4-dinitrophenylhydrazone-derivatized protein were loaded onto 12% polyacrylamide gels, followed by electrophoresis and electrophoretic transfer of protein to nitrocellulose membranes, according to the Western blot procedures described above. Optical density values of individual bands in each lane were summed to obtain a total value.

**Statistical analyses.** For dependent measures, with the exception of contractile function and body mass (BM), a 2 × 2 analysis of variance (ANOVA) was used to compare groups. All dependent measures were screened for normality and homogeneity of variance, and no dependent measure failed to meet the assumptions. In cases where the overall model was significant, specific contrasts were conducted to determine the actual nature of the differences between groups with a Bonferroni correction to account for error rate inflation as a result of multiple comparisons. For the analysis of changes in BM following surgery, a 2 × 2 × 2 repeated-measures ANOVA was used to determine whether changes in BM differed across groups. For analysis of contractile function, a repeated-measures ANOVA was used to examine changes in function across different frequency levels. For these analyses, Mauchly’s criterion was calculated to determine whether the data violated the assumption of homogeneity of variance, and corrected results (Greenhouse-Geiser corrected significance values) are reported for any analysis that violated these assumptions. Overall multivariate significance was established using Wilk’s lambda, and significant group differences were further investigated using specific contrasts with Bonferroni correction. As with the ANOVAs, any significant effects were further investigated using specific contrasts with a Bonferroni correction to account for multiple group comparisons. Values are reported as mean ± SE. A significance criterion of ≤0.05 was selected for all statistical analyses.

**RESULTS**

**Animal BM and MM.** Animal BM values are displayed in Table 1. At baseline and 7 days postsurgery, BM of MT−/−-mice was greater than that of WT mice. Following laminectomy surgery, there was no significant change in BM of WT lami or MT−/−-lami animals. As anticipated, animals in the SCT groups lost BM during the 7-day recovery period, but the percent difference between WT trans and MT−/− trans mice was not different.

Soleus MM was obtained 7 days postsurgery and is displayed in Table 1. To validate our surgical control procedure (i.e., laminectomy), we compared MM and MM-to-BM ratio values of WT lami animals to a group of WT nonsurgical controls (n = 3). Importantly, no difference in MM or MM-to-BM ratio values between WT nonsurgical controls and WT lami animals was observed (data not shown). Soleus MM of animals in the MT−/−-lami group was greater than that of WT lami animals, which is consistent with larger BM of the MT−/− animals. SCT resulted in a lower soleus MM of both SCT groups compared with respective laminectomy groups. As expected, MM-to-BM ratios declined as a result of SCT, and similar changes were observed between transected groups, with no difference observed between laminectomy groups.

**Soleus contractile properties.** Soleus muscle twitch and tetanus contractile properties are presented in Table 2. Absolute twitch force of muscles from MT−/− trans animals was lower than that of MT−/− surgical controls, and normalized twitch force (N/cm²) of MT−/− trans was lower compared with WT trans animals. No significant difference in time to peak tension or half-relaxation time was observed between groups. Muscles from MT−/− trans animals exhibited lower maximal isometric tetanic tension compared with MT−/− lami animals. Soleus muscles from SCT animals demonstrated elevated twitch-to-tetanus ratio values compared with muscles from animals in the laminectomy groups. Notably, maximal specific tension (sP₀) of soleus muscles from MT−/− trans animals was lower compared with the MT−/− lami group.

The soleus force frequency response is illustrated in Fig. 1. To ensure that the laminectomy procedure did not compromise muscle function, we assessed the force-frequency response of soleus muscles from a group of WT nonsurgical controls (n = 3). Importantly, there were no differences between the WT nonsurgical controls and WT lami animals at any of the stimulation frequency levels, as well as no significant interaction between group and stimulation frequency level (data not shown). No difference in force per CSA was observed between WT lami and MT−/− lami groups at any stimulation frequency.

### Table 1. Body mass and muscle mass

<table>
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<tr>
<th></th>
<th>n</th>
<th>BM Pre, g</th>
<th>BM Post, g</th>
<th>BM Loss, %</th>
<th>Soleus Mass, mg</th>
<th>MM/BM Ratio</th>
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<td>WT</td>
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<tr>
<td>Lami</td>
<td>7</td>
<td>30.1 ± 0.8</td>
<td>30.1 ± 0.9</td>
<td>0.0 ± 0.9</td>
<td>5.6 ± 0.2</td>
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<td>Trans</td>
<td>12</td>
<td>29.3 ± 0.6</td>
<td>26.3 ± 0.5‡</td>
<td>10.1 ± 2.0†</td>
<td>4.3 ± 0.1*</td>
<td>0.152 ± 0.006*</td>
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<td>MT−/−</td>
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<tr>
<td>Lami</td>
<td>5</td>
<td>33.8 ± 1.8‡</td>
<td>32.4 ± 1.1‡</td>
<td>4.2 ± 1.4</td>
<td>6.7 ± 0.4‡</td>
<td>0.210 ± 0.011</td>
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<tr>
<td>Trans</td>
<td>12</td>
<td>33.6 ± 0.5‡</td>
<td>29.5 ± 0.4‡</td>
<td>12.4 ± 1.5</td>
<td>4.7 ± 0.7*</td>
<td>0.160 ± 0.006*</td>
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Values are means ± SE; n, no. of animals. WT, wild-type; MT−/−, metallothionein (MT)-1/MT-2 null; Lami, laminectomy; Trans, spinal cord transection; BM, body mass; Pre, presurgery; Post, 7 days postsurgery; MM, muscle mass. ‡Different from WT; *different from Lami; †BM Post different from BM Pre: P ≤ 0.05.

### Table 2. Soleus contractile properties

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<td>Twitch</td>
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<tr>
<td>P₀,mN</td>
<td>25.8 ± 3.3</td>
<td>23.0 ± 1.2</td>
<td>32.5 ± 3.3</td>
<td>22.7 ± 1.5*</td>
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<tr>
<td>P₀,N/cm²</td>
<td>4.0 ± 0.4</td>
<td>5.0 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.2†</td>
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<td>TPF, ms</td>
<td>20.7 ± 1.9</td>
<td>21.6 ± 2.0</td>
<td>21.2 ± 1.0</td>
<td>21.1 ± 1.0</td>
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<td>½RT, ms</td>
<td>23.3 ± 1.2</td>
<td>25.0 ± 2.0</td>
<td>22.8 ± 1.5</td>
<td>24.9 ± 1.6</td>
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<td>Tetanus</td>
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<tr>
<td>P₀,mN</td>
<td>145.7 ± 12.8</td>
<td>103.1 ± 4.6</td>
<td>186.9 ± 19.3</td>
<td>109.7 ± 6.8*</td>
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<td>sP₀, N/cm²</td>
<td>22.9 ± 1.5</td>
<td>22.1 ± 0.6</td>
<td>23.5 ± 1.0</td>
<td>18.9 ± 1.0*</td>
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<td>P₀/P₀</td>
<td>0.175 ± 0.009</td>
<td>0.227 ± 0.008*</td>
<td>0.174 ± 0.007</td>
<td>0.207 ± 0.004*</td>
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Values are means ± SE; n, no. of animals. P₀, peak twitch tension; TPF, time to peak twitch tension; ½RT, half-relaxation time; P₀,mN, maximal isometric tetanic tension; sP₀, maximal specific tension; P₀/P₀, twitch-to-tetanus ratio. *Different from Lami; †different from respective WT group: P ≤ 0.05.
between 1 and 200 Hz. At low frequency of stimulation (i.e., 15 Hz), the WT trans group demonstrated greater normalized force compared with the MT\(^{-/-}\) trans group. At high-stimulation frequencies (i.e., 150 and 200 Hz), MT\(^{-/-}\) trans animals demonstrated impaired normalized force values compared with MT\(^{-/-}\) laminectomy animals.

**Protein expression of antioxidant enzymes.** Protein expression levels of Cat, MnSOD, and Cu-ZnSOD were assessed to determine whether MT deficiency resulted in altered expression of key antioxidant enzymes. Since MT is localized in the cytosol and organelles, including mitochondria (reviewed in Ref. 16), it was hypothesized that MT deficiency would result in elevated expression of cytosolic and mitochondrial antioxidant enzymes. We anticipated that an elevation in Cat, MnSOD, and Cu-ZnSOD proteins could point to a compensatory antioxidant response to counteract MT deficiency. However, no difference in the arbitrary optical density values of Cat (\(P = 0.10\)), MnSOD (\(P = 0.49\)), or Cu-ZnSOD (\(P = 0.24\)) was observed between any of the groups.

**Oxidative injury.** Oxidative injury was assessed by measurement of 4-HNE protein adducts and protein carbonyls. Representative images of 4-HNE protein carbonyls are shown in Fig. 2, A and C, respectively. 4-HNE is a reactive aldehyde formed by the action of reactive oxygen species on polyunsaturated fatty acids (43). It was hypothesized that the greatest increase in 4-HNE levels following SCT would be observed in the MT\(^{-/-}\) animals. As illustrated in Fig. 2B, SCT led to elevated 4-HNE levels in the soleus of MT\(^{-/-}\) trans animals compared with MT\(^{-/-}\) laminectomy animals. The difference in 4-HNE expression between WT laminectomy and WT trans groups did not reach statistical significance. Protein carbonyls were measured as an index of protein oxidative injury, but levels were not different between experimental groups (Fig. 2D).

**Soleus muscle fiber CSA and distribution.** Mean type I and type Ila muscle fiber CSA values are illustrated in Fig. 3. Representative images of soleus muscle obtained from a laminectomy and transection animal are shown in Fig. 3A. Both type I and type Ila fibers were smaller in soleus muscles from SCT animals compared with laminectomy animals (Fig. 3B). No difference in fiber CSA was detected between WT and MT\(^{-/-}\) animals. Type I, Ila, and Ix/Iib fibers were counted and expressed as a percentage of the total fiber count. No difference in fiber distribution was observed between any of the groups (Fig. 3C). Fiber CSA of Ix/Iib fibers was not assessed due to the low number of unstained fibers.

**DISCUSSION**

In recent years, much emphasis has been directed toward unraveling mechanisms that underlie how decreases in skeletal muscle activity lead to atrophy and contractile dysfunction. Of particular interest has been discerning the role of stress proteins as mediators of skeletal muscle adaptations in response to disuse. The current experiment provides novel insight into the putative role of MT in mediating skeletal muscle adaptations following acute spinal cord injury. This is the first study to utilize a mouse model deficient in MT-1 and MT-2 proteins to study the contractile and atrophy response of the soleus to acute SCT. The results suggest that deficiency in MT-1 and MT-2 proteins does not alter the extent of soleus muscle atrophy, but is a contributing factor to the impairment of soleus muscle contractile function following acute spinal cord injury.

Over the 7-day period following SCT, animal BM declined ~11% in WT trans and MT\(^{-/-}\) trans animals, with no significant change observed in laminectomy animals. In all groups, food intake was carefully monitored, and the animals were provided with a supplemented diet (see Experimental Methods) to help achieve an adequate caloric intake, both before and after surgery. In response to these efforts, the amount of BM loss following SCT was noted to be less than one-half of the BM loss reported to occur in a recent study employing SCT in mice over the same length of time (27). As expected, 7 days following the onset of injury, significant atrophy of the soleus was observed in both SCT groups. While it was anticipated that MT deficiency would exacerbate the atrophy response of the soleus following SCT, this outcome was not observed. The MM-to-BM ratio showed no difference between MT\(^{-/-}\) and WT animals and was reduced to a similar degree in both groups of SCT animals. Moreover, the observation of similar reductions in the CSA of type I and type Ila fibers among SCT groups is also consistent with the pattern of MM loss. Collectively, our data indicate that MT deficiency does not alter the atrophy response of the soleus following acute SCT.

Despite the lack of influence of MT deficiency on the extent of soleus muscle atrophy, muscles from MT\(^{-/-}\) mice did exhibit impaired maximal force generation per CSA (i.e., sPo) following SCT compared with the response of muscles from MT\(^{-/-}\) surgical controls. Of further interest was the observation of similar normalized force values of MT\(^{-/-}\) lami and WT lami animals across a broad range of stimulation frequencies, a finding that suggests MT deficiency per se does not alter force production of unfatigued muscle. Consistent with previous data obtained from mice (27) and rats (6, 30, 41), SCT did not lead to increased soleus sPo of WT trans animals. In fact, 1 yr following SCT, the sPo of rat soleus has been shown to increase by >100% (30). This is in contrast to a decreased sPo that is routinely reported to occur in other models of muscle disuse, such as immobilization and hindlimb suspension (15,
Factors that may explain the maintenance or increase in $sP_o$ following SCT include MHC phenotype shifts and changes in isoform expression of thick-filament proteins (e.g., myosin light chain and C-protein) (29, 30, 41). The present data do not support MHC phenotype shifts as a potential factor in the maintenance of $sP_o$ in WT trans animals, since no difference in the MHC phenotype was observed in the 1-wk period following surgery. This finding is consistent with data obtained from rats in which transformation of soleus fibers from a slow to fast phenotype was reported to begin around 10-days post-transection (14). Differences in the isoform expression of thick-filament proteins remain a potential explanation, but, to our knowledge, an association between MT deficiency and expression of thick-filament proteins has not been established.

The decrease in maximal force-generating ability per CSA ($sP_o$) of soleus muscles from MT$^{-/-}$ trans mice compared with muscles from MT$^{-/-}$ surgical controls could have been the result of factors that include the presence of abnormal contractile proteins, impaired membrane excitability, and/or altered calcium handling. Alterations in the MHC phenotype or concentration of contractile proteins do not appear to be factors that would explain the decrease in soleus $sP_o$ of MT$^{-/-}$ trans animals, since 1) no change in the MHC phenotype was observed; and 2) a decrease in the concentration of myofibrillar protein may be expected to reduce force generation at high...

**Fig. 2.** Oxidative injury assessed by Western blot for 4-hydroxynoneal (4-HNE) protein adducts and protein carbonyls. Representative Western blot image of 4-HNE (A) and protein carbonyls (C) are shown. Numbers on the left indicate molecular mass (in kDa). Samples are in the following order (from left to right): WT lami, WT trans, MT$^{-/-}$ lami, and MT$^{-/-}$ trans. Arbitrary optical density values for 4-HNE (B) and protein carbonyls (D) are shown. Open bar, WT lami ($n = 5$); open hatched bar, WT trans ($n = 7$); shaded bar, MT$^{-/-}$ lami ($n = 5$); and shaded hatched bar, MT$^{-/-}$ trans ($n = 8$) animals. Values are means ± SE. *MT$^{-/-}$ trans greater than MT$^{-/-}$ lami: $P < 0.05$. 

40, 46).
and low-stimulation frequencies. The presence of abnormal contractile proteins cannot be ruled out, but lack of definitive evidence of oxidative stress lessens the likelihood of redox-modulated protein modifications. Alternatively, we speculate that the reduced sPo of soleus muscles from MT−/− trans mice may have been the result of impairments in membrane excitability and/or calcium handling. For example, membrane excitability may have been affected by elevated levels of 4-HNE. Superfusion of isolated rat ventricular myocytes with 4-HNE results in early inhibition of inward rectifier K+ current, prolongation of the action potential, and loss of membrane excitability (3). Additionally, MT may play a role in the regulation of intracellular calcium under pathologic conditions. For instance, reductions in intracellular Ca2+ rise and clearance rates in cardiomyocytes obtained from mice fed a chronic high-fat diet were reversed by cardiac-specific overexpression of MT in mice fed the same high-fat diet (12). The rectification of Ca2+ handling by MT overexpression was also associated with lower production of reactive oxygen species, restoration of contractile function, and reversal of high-fat-induced mitochondrial damage and reduced mitochondrial density (12). However, the suggestion of impaired Ca2+ handling in muscles from MT−/− animals following SCT would be inconsistent with the lack of change noted in time to peak tension and half-relaxation time parameters, which clearly indicates the need for further study to elucidate the underlying mechanism responsible for the reduction in sPo.

It was anticipated that deficiency of MT would lead to exacerbated oxidative injury following SCT. Although the greater level of 4-HNE detected in soleus muscles from MT−/− trans animals compared with muscles from MT−/− surgical controls revealed a greater degree of lipid peroxidation, there was no definitive evidence of oxidative stress, since no change in the levels of protein carbonyls or key antioxidant proteins was observed. It is conceivable that lack of MT in the MT−/− trans animals led to greater lipid peroxidation as a result of lower cellular zinc levels. MT deficiency may compromise tissue zinc status and limit zinc availability under conditions of stress, thus leading to increased tissue damage (26). Importantly, zinc has been shown to stabilize membrane integrity and inhibit lipid peroxidation (7, 8). Therefore, MT−/− trans animals may have experienced altered zinc levels that could have contributed to the increase in 4-HNE. It was also hypothesized that lack of MT would be associated with upregulation of key antioxidant defense proteins in response to SCT-mediated muscle disuse. Contrary to our hypothesis, this outcome was not observed. An important study by Conrad et al. (9) utilized MT+/+ and MT−/− mice to investigate the antioxidant and oxidative injury response of liver following exposure of the mice to oxidative stress. Notably, the activities and mRNA expression of key antioxidant enzymes were not different between MT+/+ and MT−/− animals in response to oxidative stress. Although the measurement of antioxidant enzyme activities in the present study would have provided a more definitive assessment of antioxidant function, the lack of change in antioxidant protein levels is consistent with these previous findings (9), in that antioxidant upregulation does not appear to occur in conjunction with MT deficiency in the basal state or under conditions of stress. In the paper by Conrad et al., it should also be noted that, following exposure of the mice to oxidative stress, no difference in markers of liver oxidative injury (e.g., DNA and protein oxidation, lipid peroxidation) was observed between MT+/+ and MT-deficient strains (9).

Everything considered, despite similarities and differences between the findings of Conrad et al. and the present study, outcomes may ultimately be dependent on the method of stress induction and the tissue/cell type studied, particularly since

Fig. 3. Muscle fiber cross-sectional area (CSA) and fiber-type distribution. Measurements were obtained from WT lami (n = 4), WT trans (n = 4), MT−/− lami (n = 4), and MT−/− trans (n = 4) animals. A: representative tissue sections stained for dystrophin (red), type I (blue), type IIa (green), and type IIx/b (unstained) fibers from a laminectomy (left) and transection (right) animal. Scale bar = 50 μM. B: mean soleus muscle fiber CSA. C: average percent distribution of type I, type IIa, and type IIx/b soleus muscle fibers. Values are means ± SE. *Different from respective laminectomy group: P ≤ 0.05.
skeletal muscle possesses a lower antioxidant status compared with that of liver (9, 17, 20). Clearly, additional work must be conducted to delineate the absolute impact of MT deficiency on skeletal muscle antioxidant capacity and oxidative stress.

Perspectives and significance. This is the first experiment to utilize an established MT-1/MT-2 null mouse model to examine the impact of MT deficiency on the adaptation of soleus muscle subjected to disuse. Before this investigation, information pertaining to the potential role of MT in mediating adaptations of skeletal muscle to disuse had been limited to the characterization of MT mRNA and protein expression patterns. In this experiment, it was hypothesized that lack of MT-1 and MT-2 proteins (MT−/−) would augment the degree of soleus muscle atrophy and oxidative injury following spinal cord injury, as well as exacerbate the loss of muscle force-generating ability. Although MT deficiency did not alter the atrophy response of the soleus following the 1-wk period after induction of spinal cord injury, the results do suggest that MT may play an important role in the regulation of soleus muscle functional status. While the mechanisms(s) responsible for the observed muscle force deficits in MT-deficient mice following spinal cord injury were not identified, it remains to be determined if this effect was the result of a compromised redox status, zinc status, or other factor(s). Future research should be aimed at delineating the potential mechanism(s) responsible for the specific force deficits, as well as examine the importance of MT in mediating skeletal muscle adaptations in response to other forms of stress.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


