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

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DeRuisseau LR, Recca DM, Mogle JA, Zoccolillo M, DeRuisseau KC. 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 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−/− trans 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.

metal atrophy; oxidative stress; spinal cord transection; antioxidant; stress protein

METALLOTHIONEIN (MT) IS A SMALL MOLECULAR WEIGHT 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 UBQUITOUSLY 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 SULFHYDRL

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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).

Myofiber CSA. Sections from frozen muscles embedded in optimum cutting temperature compound were cut at 10 μm using a cryostat, mounted on slides, and air dried for 30 min, followed by incubation in PBS containing 0.5% Triton X-100. Sections were rinsed in PBS and simultaneously exposed to dystrophin (Thermo Fisher Scientific, Fremont, CA), myosin heavy chain type (MHC) I (A4844; DSHB, Iowa City, IA) and MHC type IIa (N2.261; DSHB) antibodies in a dark, humid chamber at room temperature for 1 h. Sections were rinsed in PBS and exposed to TRIT-C goat anti-rabbit (Invitrogen, Carlsbad, CA), Alexa Fluor 350 goat anti-mouse (Molecular Probes, Eugene, OR), and FITC goat anti-mouse (Invitrogen) secondary antibodies diluted in PBS in a dark chamber at room temperature for 1 h. Sections were washed in PBS and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). After mounting, slides were coverslipped and sealed for viewing via an upright Zeiss AxiosImager fluorescence microscope. With this technique I fibers stain blue, type IIa fibers stain green, and dystrophin stains red. Unstained fibers were assumed to be either type IIx or type IIb (IIx/b). Images were obtained using a Zeiss AxiosCam MRC digital camera at ×10 magnification, and myofiber CSA (μm²) was determined on an average of 125 type I and type IIa fibers using Zeiss AxioVision software (version 4; Carl Zeiss). Fiber-type proportion was determined from counts of type I, type IIa, and type IIX/IIb fibers and expressed as a percentage of the total number of fibers.

Western blot assessment of antioxidant proteins and 4-hydroxynonenal 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; protease inhibitor cocktail (Sigma, St. Louis, MO)] and centrifuged at 10,000 g for 10 min at 4°C. Protein content of the soluble fraction was assessed by the method of Bradford (Sigma, St. Louis, MO). Proteins (20–30 μg) were individually separated by SDS-PAGE via 12% polyacrylamide gels containing 0.1% SDS. Following electrophoresis, the proteins were transferred to nitrocellulose membranes (100 V for 3 h) followed by staining with Ponceau S and visually inspected for equal protein loading/transfer. The membranes were then washed and blocked in PBS-Tween buffer containing 5.0% skim milk and 0.05% Tween for 2 h and subsequently incubated with a primary antibody directed against catalase (Cat) (219010; CalBiochem, San Diego, CA), manganese superoxide dismutase (MnSOD) (SOD110-D; Assay Designs, Ann Arbor, MI), copper-zinc superoxide dismutase (Cu-ZnSOD) (NB100-60944; Novus Biologicals, Littleton, CO), or 4-hydroxynonenal (4-HNE) (MAB3049; R&D Systems, Minneapolis, MN). Primary antibodies were diluted 1:1,000 in blocking buffer and applied to the membranes overnight at 4°C. This step was followed by incubation with a horseshad peroxidase-antibody conjugate (1:5,000) directed against the primary antibody for 45 min. The membranes were washed and subsequently treated with chemiluminescent reagents (Thermo Scientific, Rockford, IL) and exposed to light-sensitive film. The film was subsequently digitized and analyzed using ImageJ software (1). Optical density values of individual 4-HNE bands within each lane were summed to obtain a total value.

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 manufacturer’s instructions. Briefly, 15 μg of soluble protein were reacted

(TM "lami," n = 7), wild-type transaction (TM trans, n = 12), MT-/- laminectomy (MT-/- lami, n = 5), and MT-/- transaction (MT-/- trans; n = 12).

Laminectomy and SCT at thoracic level T9. Animals were anesthetized 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 was exposed. The dorsal spinous processes and vertebrae were cleaned with a microcurette, and a laminectomy was performed using microrongeurs 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 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 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 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 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 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 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 3 mm after this procedure. The musculature was sutured closed (4-0 silk) in layers, followed by closure of the skin (4-0 silk).
with 2,4-dinitrophenylhydrazine for 15 min. Following the reaction process, 10 μg of 2,4-dinitrophenylhydrazone-derivated 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 $2 \times 2$ analysis of variance (ANOVA) was used to compare groups. All dependent measures were screened for normality and heterogeneity 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 $2 \times 2 \times 2$ 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 $\pm$ SE. A significance criterion of $\leq 0.05$ 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/cm$^2$) 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; $sP_{\text{m}}$, maximal specific tension; $P_t/P_o$, twitch-to-tetanus ratio.

*Different from Lami; †different from respective WT group; $P \leq 0.05$.

Table 2. Soleus contractile properties

<table>
<thead>
<tr>
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<th>WT</th>
<th>MT$^{-/-}$</th>
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<tbody>
<tr>
<td></td>
<td>Lami</td>
<td>Trans</td>
</tr>
<tr>
<td>$n$</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Twitch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_t$, mN</td>
<td>25.8±3.3</td>
<td>23.0±1.2</td>
</tr>
<tr>
<td>$P_t$, N/cm$^2$</td>
<td>4.0±0.4</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>TPF, ms</td>
<td>20.7±1.9</td>
<td>21.6±2.0</td>
</tr>
<tr>
<td>$1/2RT$, ms</td>
<td>23.3±1.2</td>
<td>25.0±2.0</td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_t$, mN</td>
<td>145.7±12.8</td>
<td>101.3±4.6</td>
</tr>
<tr>
<td>$sP_{\text{m}}$, N/cm$^2$</td>
<td>22.9±1.5</td>
<td>22.1±0.6</td>
</tr>
<tr>
<td>$P_t/P_o$, twitch-to-tetanus ratio</td>
<td>0.175±0.009</td>
<td>0.227±0.008*</td>
</tr>
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</table>

Values are means $\pm$ SE; $n$, no. of animals. $P_t$, peak twitch tension; $P_t/P_o$, twitch to tetanus tension; $1/2RT$, half-relaxation time; $sP_{\text{m}}$, maximal specific tension; $P_t/P_o$, twitch-tetanus ratio. *Different from Lami; †different from respective WT group; $P \geq 0.05$.

Table 1. Body mass and muscle mass

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>MT$^{-/-}$</th>
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<tr>
<td></td>
<td>Lami</td>
<td>Trans</td>
</tr>
<tr>
<td>$n$</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>BM, g</td>
<td>30.1±0.8</td>
<td>29.3±0.6</td>
</tr>
<tr>
<td>BM, g</td>
<td>30.1±0.9</td>
<td>26.3±0.5*</td>
</tr>
<tr>
<td>BM, %</td>
<td>00.0±0.9</td>
<td>10.1±2.0†</td>
</tr>
<tr>
<td>SO, mg</td>
<td>5.6±0.2</td>
<td>4.3±0.1*</td>
</tr>
<tr>
<td>MM/BM</td>
<td>0.187±0.005</td>
<td>0.152±0.006*</td>
</tr>
</tbody>
</table>

Values are means $\pm$ 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. $\dagger$Different from WT; $\dagger$different from Lami; †BM Post different from BM Pre; $P \leq 0.05$. 

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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-/- lami 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 carboxyls. Representative images of 4-HNE and protein carboxyls 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-/- lami animals. The difference in 4-HNE expression between WT lami and WT trans groups did not reach statistical significance. Protein carboxyls 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 IIA 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 IIA 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, IIA, and IIX/b 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 IIX/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 IIA 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 impaired 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 sPo 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 sPo 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 (sPo) 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 sPo 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-
soleus muscles from MT−/− trans animals led to greater lipid peroxidation as a result of lower cellular zinc levels. MT deficiency may compromise 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-1/MT-2-null (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

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 sPă 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 pathological 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 disease. Contrary to our hypothesis, this outcome was not observed. An important study by Conrad et al. (9) utilized MT+/+ and MT-1/MT-2-null (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
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 mechanism(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


