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MnSOD deficiency results in elevated oxidative stress and decreased mitochondrial function but does not lead to muscle atrophy during aging

Michael S. Lustgarten[1,4], **Youngmok C. Jang**[2,4], **Yuhong Liu**[2], **Wenbo Qi**[4], **Yuejuan Qin**[3], **Patricia L. Dahia**[3], **Yun Shi**[1], **Arunabh Bhattacharya**[2,4], **Florian L. Muller**[2], **Takahiko Shimizu**[6], **Takuji Shirasawa**[6], **Arlan Richardson**[2,4,5], and **Holly Van Remmen**[2,4,5]

[1] Department of Physiology, University of Texas Health Science Center at San Antonio

[2] Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio

[3] Department of Medicine, Hematology and Medical Oncology, University of Texas Health Science Center at San Antonio

[4] Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio

[5] South Texas Veterans Health Care System, Audie L. Murphy Division

[6] Research Team for Molecular Biomarkers-Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Summary

In a previous study, we reported that a deficiency in MnSOD activity (approximately 80% reduction) targeted to type IIB skeletal muscle fibers was sufficient to elevate oxidative stress and to reduce muscle function in young adult mice (*TnIFastCreSod2*$^{fl/fl}$ mice). In the present study, we used *TnIFastCreSod2*$^{fl/fl}$ mice to examine the effect of elevated oxidative stress on mitochondrial function and to test the hypothesis that elevated oxidative stress and decreased mitochondrial function over the lifespan of the *TnIFastCreSod2*$^{fl/fl}$ mice would be sufficient to accelerate muscle atrophy associated with aging. We found that mitochondrial function is reduced in both young and old *TnIFastCreSod2*$^{fl/fl}$ mice, when compared with control mice. Complex II activity is reduced by 47% in young and by ~90% in old *TnIFastCreSod2*$^{fl/fl}$ mice, associated with reduced levels of the catalytic subunits for complex II, SDHA and SDHB. Complex II-linked mitochondrial respiration is reduced by approximately 70% in young *TnIFastCreSod2*$^{fl/fl}$ mice. Complex II-linked mitochondrial ATP production is reduced by 39% in young and was found to be almost completely absent in old *TnIFastCreSod2*$^{fl/fl}$ mice. Furthermore, in old *TnIFastCreSod2*$^{fl/fl}$ mice, aconitase activity is almost completely abolished; mitochondrial superoxide release remains greater than 2-fold elevated; and oxidative damage (measured as F_2 isoprostanes) is increased by 30% relative to age-matched controls. These data show that despite elevated skeletal muscle-specific mitochondrial oxidative stress, oxidative damage and complex II-linked mitochondrial dysfunction, age-related muscle atrophy was not accelerated in old *TnIFastCreSod2*$^{fl/fl}$ mice, suggesting mitochondrial oxidative stress may not be causal for age-related muscle atrophy.

Corresponding author: Holly Van Remmen, Ph.D., The Sam and Ann Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, 15355 Lambda Drive, San Antonio, Texas 78245, Fax: (210) 562-6110, vanremmen@uthscsa.edu.

Introduction

Elevated oxidative stress (Lopez *et al.*, 2000; Wanagat *et al.*, 2001; Fulle *et al.*, 2004, Mansouri *et al*., 2006; Muller *et al.*, 2007) and decreased mitochondrial function (Short *et al.*, 2005, Marcinek *et al.*, 2005; Mansouri *et al.*, 2006) have been shown to be associated with age-related skeletal muscle atrophy yet, a causal role for increased oxidative stress and mitochondrial dysfunction has not been established. Knockout mouse models with alterations in mitochondrial antioxidant defense provide a mechanism for directly testing the effect of elevated mitochondrial oxidative stress and mitochondrial dysfunction on skeletal muscle atrophy. Mice deficient in MnSOD, the first line of antioxidant defense against superoxide radicals in the mitochondrial matrix, are an excellent model to study the effects of elevated mitochondrial oxidative stress. However, complete deletion of *Sod2*$^{-/-}$ leads to lethality within days to just a few weeks depending on genetic background (Huang *et al.*, 2006), and therefore *Sod2*$^{-/-}$ are not a useful model for aging studies.

To circumvent the lethality of complete deficiency of MnSOD, investigators have generated mouse models with targeted deletion of MnSOD in specific tissues using Cre-Lox approaches. Young mice with combined heart and skeletal muscle deletion of *Sod2* (H/M-*Sod2*$^{-/-}$ mice, Nojiri *et al.*, 2006), or, skeletal muscle-specific MnSOD knockout mice (muscle-*Sod2*$^{-/-}$; Kuwahara *et al.*, 2010) have been shown to have elevated mitochondrial oxidative stress in the affected tissues. Despite the elevated mitochondrial oxidative stress in the skeletal muscle of these two mouse models, there was no indication of muscle atrophy in either animal model (Nojiri *et al.*, 2006; Kuwahara *et al.*, 2010). Similarly, in a previous study, we reported that young adult *TnIFastCreSod2*$^{fl/fl}$ mice that have a 70% reduction in MnSOD content specifically in type IIB fibers, elevated mitochondrial oxidative stress and oxidative damage, show no loss of muscle mass (Lustgarten *et al.*, 2009). Thus, collective evidence from the H/M-*Sod2*$^{-/-}$, muscle-*Sod2*$^{-/-}$, and *TnIFastCreSod2*$^{fl/fl}$ mice suggests that elevated mitochondrial oxidative stress and mitochondrial dysfunction are not sufficient to initiate muscle atrophy, at least in young mice. However, it is possible that over the lifespan chronic elevation of mitochondrial dysfunction and oxidative stress might contribute to muscle atrophy in older mice. To address this question, we asked whether elevated mitochondrial dysfunction and oxidative stress resulting from MnSOD deficiency targeted to type IIB enriched skeletal muscle in *TnIFastCreSod2*$^{fl/fl}$ mice leads to an accelerated loss of muscle mass during aging.

Results

Mitochondrial function is reduced in young TnIFastCreSod2$^{fl/fl}$ mice

We have previously shown that mitochondria isolated from type IIB enriched skeletal muscle in young *TnIFastCreSod2*$^{fl/fl}$ mice have a greater than 80% decrease in MnSOD activity, elevated intra-mitochondrial superoxide, elevated mitochondrial superoxide release, and increased lipid oxidative damage, relative to wild type (Lustgarten *et al.*, 2009). However, the role of elevated type IIB enriched skeletal muscle mitochondrial oxidative stress on mitochondrial function in *TnIFastCreSod2*$^{fl/fl}$ mice was not addressed. To determine the effect of the targeted reduction of MnSOD in type IIB muscle fibers on muscle mitochondrial function, we measured mitochondrial oxygen consumption and ATP production in skeletal muscle mitochondria isolated from type IIB enriched skeletal muscle in young adult (6–8 month) *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. We found no significant difference in the glutamate plus malate (GM) supported basal rate of mitochondrial oxygen consumption (in the absence of ADP) in mitochondria isolated from type IIB enriched skeletal muscle from *TnIFastCreSod2*$^{fl/fl}$ when compared with wild type mice (Figure 1A). GM-stimulated maximal respiration (in the presence of exogenously added ADP) was reduced by 32% in mitochondria isolated from type IIB enriched muscle from

TnIFastCreSod2$^{fl/fl}$ mice, but this difference was not statistically significant (Figure 1B). Succinate plus rotenone (SR)-linked basal and maximal respiration were reduced by 65% (Figure 1C) and 74% (Figure 1D), respectively, in type IIB enriched muscle from *TnIFastCreSod2*$^{fl/fl}$ mice when compared with wild type. The rate of mitochondrial ATP production by mitochondria isolated from type IIB enriched skeletal muscle respiring on several substrates including GM (Figure 1E), α-ketoglutarate (Figure 1F) and α-glycerol phosphate (Figure 1G) was not significantly different between *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. In contrast, the rate of mitochondrial ATP production with SR used as substrate was reduced by 39% in type IIB enriched muscle from *TnIFastCreSod2*$^{fl/fl}$ mice (Figure 1E), indicating a complex II-specific defect in type IIB enriched skeletal muscle from *TnIFastCreSod2*$^{fl/fl}$. Thus, mitochondrial function with complex II-linked substrate was altered in young mice with a targeted reduction of MnSOD.

Activities of Complex I and II are reduced in TnIFastCreSod2$^{fl/fl}$ mice

The electron transport chain (ETC), consisting of 5 protein complexes: complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinol-cytochrome c reductase), complex IV (cytochrome c oxidase) and complex V (ATP synthase) is located in the inner mitochondrial membrane. Therefore, the lack of MnSOD could potentially increase oxidative stress induced damage to the ETC complexes. As shown in Figure 2, the activities of complexes I and II were reduced by 32% and 47%, respectively, in mitochondria isolated from type IIB enriched skeletal muscle in *TnIFastCreSod2*$^{fl/fl}$ compared to muscle mitochondria from wild type. Activities of complex III and IV were not different in the two mouse models. In addition, there was no difference in the activities of complexes I, II, III, or IV in homogenates of type I fiber enriched skeletal muscle (soleus) from *TnIFastCreSod2*$^{fl/fl}$ or wild type mice (*data not shown*). We confirmed the changes in ETC activity using histological analysis of gastrocnemius muscle sections. As shown in Figure 3, complex IV activity is not changed, but clear zones indicative of reduced complex II activity are evident in muscle from *TnIFastCreSod2*$^{fl/fl}$ mice, when compared to muscle from wild type mice.

SDHA and SDHB protein content are reduced but levels of SdhA and SdhB mRNA are not significantly different in young TnIFastCreSod2$^{fl/fl}$ mice

Complex II is composed of two hydrophilic (SDHA and SDHB) and two hydrophobic membrane localized subunits (SDHC and SDHD). To determine whether the reduced activity of complex II is associated with a reduction in the level of complex II protein, we measured the level of SDHA and SDHB in mitochondria isolated from type IIB enriched muscle in *TnIFastCreSod2*$^{fl/fl}$ and wild type control mice. SDHA and SDHB protein content were reduced by 32% and 70%, respectively (Figure 4A-C). SDHA and SDHB protein levels were not different in homogenates of type I fiber enriched soleus muscles isolated from *TnIFastCreSod2*$^{fl/fl}$ and wild type mice (*data not shown*). We also measured *SdhA* and *SdhB* mRNA content to determine whether the deficit in SDHA and SDHB protein content found in *TnIFastCreSod2*$^{fl/fl}$ was associated with a decrease in gene transcription. The mRNA content for *SdhA* and *SdhB* found in type IIB enriched skeletal muscle was not significantly different in muscle isolated from *TnIFastCreSod2*$^{fl/fl}$ and wild type mice (Figure 4D).

Activities of other TCA cycle enzymes are not decreased in young TnIFastCreSod2$^{fl/fl}$ mice

To determine whether the reduction in the enzymatic activities of aconitase and succinate dehydrogenase (SDH) was selective, we measured the activity of the mitochondrial matrix-localized citric acid cycle (TCA) enzymes citrate synthase, isocitrate dehydrogenase, malate dehydrogenase and fumarase. No significant difference was found for the enzymatic activity of citrate synthase, isocitrate dehydrogenase, malate dehydrogenase, or fumarase in either

type IIB enriched skeletal muscle (Table I) or in the type I enriched soleus muscle (*data not shown*) when comparing the values obtained in *TnIFastCreSod2$^{fl/fl}$* with wild type mice. These data indicate that the TCA cycle enzymes aconitase and SDH are preferentially sensitive to MnSOD deficiency.

SDH is sensitive to inactivation by superoxide

Aconitase inactivation was previously shown to be due to superoxide mediated Fe-S cluster damage (Gardner *et al.*, 1995). The SDHB subunit of complex II contains three Fe-S clusters (Hagerhall 1997). Therefore, we asked whether the decrease in SDH activity found in *TnIFastCreSod2$^{fl/fl}$* muscle was related to superoxide mediated inactivation. As shown in Figure 5A, SDH activity is reduced in the presence of potassium superoxide *in vitro,* demonstrating that SDH is also susceptible to inactivation by superoxide. In contrast, the activities of isocitrate dehydrogenase (Figure 5B) and fumarase (Figure 5C) are not altered by exposure to potassium superoxide.

Oxidative stress and oxidative damage are elevated in old TnIFastCreSod2$^{fl/fl}$ mice

In a previous study, we demonstrated that type IIB enriched muscle from the *TnIFastCreSod2$^{fl/fl}$* mice had increased levels of oxidative stress as measured by reduced aconitase activity, increased superoxide release and increased oxidative damage (measured as F_2 isoprostanes; Lustgarten *et al*., 2009). We hypothesized that a lifelong reduction in MnSOD content would lead to an increase in mitochondrial matrix-localized superoxide and potentially exacerbate oxidative stress in older animals. To ask whether mitochondrial oxidative stress is elevated during aging in muscle from *TnIFastCreSod2$^{fl/fl}$* mice, we measured aconitase activity in young and old wild type and *TnIFastCreSod2$^{fl/fl}$* mice. As shown in Figure 6A, aconitase activity was reduced by approximately 90% in old (27–34 month) *TnIFastCreSod2$^{fl/fl}$* mice when compared with the value obtained in either young *TnIFastCreSod2$^{fl/fl}$* or in old wild type mice. Furthermore, aconitase activity is decreased by more than 35% during aging in wild type mice. Interestingly, the aconitase protein level is also reduced by more than 50% in type IIB enriched muscle from old *TnIFastCreSod2$^{fl/fl}$* mice when compared with young *TnIFastCreSod2$^{fl/fl}$* mice (Figure 6B). This effect is specific to type IIB enriched skeletal muscle in the *TnIFastCreSod2$^{fl/fl}$* mice as we found no significant difference in aconitase protein in Type I fiber enriched soleus muscle from old mice of either genotype (Figure 6B). These data demonstrate an increase in type IIB enriched skeletal muscle mitochondrial matrix oxidative stress during aging that occurs in old wild type mice but to a greater extent in old *TnIFastCreSod2$^{fl/fl}$* mice.

Because aconitase activity is an indirect indicator of mitochondrial matrix superoxide content, we directly measured mitochondrial superoxide release with use of electron paramagnetic resonance (EPR). Mitochondria isolated from type IIB enriched skeletal muscle from old *TnIFastCreSod2$^{fl/fl}$* or old wild type mice do not release more superoxide than mitochondria isolated from their young, genotype-matched counterparts (Figure 6C, 6D). However, mitochondria isolated from both young and old *TnIFastCreSod2$^{fl/fl}$* mice release greater than 2-fold more superoxide than mitochondria isolated from age-matched wild type mice in the presence of either GM or SR as respiratory substrates. Thus, the reduction in MnSOD results in elevated levels of superoxide that can be detected extra-mitochondrially.

To determine whether a reduction in MnSOD content leads to an increase in oxidative damage during aging in *TnIFastCreSod2$^{fl/fl}$* mice, we measured lipid peroxidation (F_2-Isoprostanes) in the tibialis anterior muscle (35% type IIB fibers) isolated from 21-month-old *TnIFastCreSod2$^{fl/fl}$* mice. We had previously shown that young adult *TnIFastCreSod2$^{fl/fl}$* mice have elevated levels of F_2- isoprostanes (Lustgarten *et al*., 2009). Here we found a

significant elevation (30%) of F_2-Isoprostanes in tibialis anterior muscle isolated from middle-aged *TnIFastCreSod2*$^{fl/fl}$ mice, when compared with the value obtained in middle-aged wild type mice (Figure 6E).

Complex II-linked ATP production, complex II activity and protein content are each reduced in old TnIFastCreSod2$^{fl/fl}$ mice

The rate of mitochondrial ATP production with GM used as substrate was not significantly different in mitochondria isolated from type IIB enriched skeletal muscle in old *TnIFastCreSod2*$^{fl/fl}$ mice, when compared with the corresponding value in age-matched wild type mice (Figure 7A). However, in response to SR as substrate, the rate of mitochondrial ATP production was reduced by 98% in old *TnIFastCreSod2*$^{fl/fl}$ mice, relative to age-matched wild type (Figure 7B). The activities of complexes I through IV were measured to investigate whether mitochondrial respiratory complex activity was impaired during aging in *TnIFastCreSod2*$^{fl/fl}$. Complex II activity was selectively reduced by 88% (Figure 7D), but the activity of the other three protein complexes was not different when comparing old mutant mice with age-matched wild type. SDHA and SDHB protein content were found to be reduced by more than 50% and 90%, respectively (Figure 7G) in type IIB enriched muscle isolated from *TnIFastCreSod2*$^{fl/fl}$ mice, when compared with age-matched wild type. In contrast, levels of SDHA and SDHB were not significantly different in homogenates of type I fiber enriched soleus muscles isolated from old *TnIFastCreSod2*$^{fl/fl}$, when compared withold wild type mice (Figure 7H).

Muscle atrophy is not greater during aging in TnIFastCreSod2$^{fl/fl}$ mice

The preceding experiments demonstrated an increase in oxidative stress and mitochondrial dysfunction in mitochondria isolated from type IIB enriched muscle in *TnIFastCreSod2*$^{fl/fl}$ mice during aging. If mitochondrial dysfunction and oxidative stress play a casual role in muscle atrophy during aging, we would predict an increase in muscle atrophy in muscle with high type IIB content (and therefore low MnSOD activity) in the *TnIFastCreSod2*$^{fl/fl}$ mice. As shown in Table II, the mass of the type IIB rich gastrocnemius muscle (normalized to body mass, G/BW) and type I rich soleus muscle (S/BW) was examined in young (3–4 months) and old (27–34 months) *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. G/BW declined significantly during aging for both wild type and *TnIFastCreSod2*$^{fl/fl}$ female but not male mice (*data not shown)*. S/BW did not change during aging for either genotype or gender during aging. Significant increases in body mass and the percentage of body fat were identified for both old wild type and *TnIFastCreSod2*$^{fl/fl}$ female, but not male mice (*data not shown)* during aging. In addition, the percentage of lean mass significantly declined during aging for both wild type and *TnIFastCreSod2*$^{fl/fl}$ female mice. However, lifelong elevated oxidative stress, oxidative damage and mitochondrial dysfunction targeted to type IIB enriched-skeletal muscle was not sufficient to further alter G/BW, S/BW, body mass, or the respective percentages of body fat and lean mass during aging in female or male (*data not shown) TnIFastCreSod2*$^{fl/fl}$, when compared to age-matched wild type mice.

Discussion

The goal of the present study was to investigate the role of chronic elevations mitochondrial oxidative stress, oxidative damage and mitochondrial dysfunction on age-related muscle atrophy in a mouse model with targeted deletion of the mitochondrial antioxidant, MnSOD in type IIB muscle fibers. The data presented in this report show that increases in mitochondrial oxidative stress and oxidative damage, and alterations in mitochondrial function caused by MnSOD deficiency in type IIB fibers does not lead to skeletal muscle fiber atrophy in either young or old mice. In young mice with a greater than 80% reduction in MnSOD activity in type IIB enriched skeletal muscle, we found reduced aconitase

activity (Lustgarten *et al.*, 2009), decreased rates of mitochondrial oxygen consumption and ATP production, and a specific defect in complex II activity and protein content. In old *TnIFastCreSod2*$^{fl/fl}$ mice, aconitase activity was further reduced, mitochondrial superoxide release was elevated, oxidative damage was increased, and mitochondrial function was decreased, but, there was no acceleration of the rate of muscle atrophy, relative to the values found in age-matched wild type mice. These results are consistent with previous studies in mice with a 50% reduction in MnSOD (*Sod2*$^{+/-}$ mice, Van Remmen *et al.*, 2001; Van Remmen *et al.*, 2003; Mansouri *et al.*, 2006), in mice deficient in MnSOD in both skeletal muscle and heart (H/M-*Sod2*$^{-/-}$, Nojiri *et al.*, 2006), and in mice lacking MnSOD in skeletal muscle (M-*Sod2*$^{-/-}$, Kuwahara *et al.*, 2010), as none of these models show increases in muscle atrophy despite elevated mitochondrial oxidative stress and mitochondrial dysfunction.

Skeletal muscle is a heterogeneous tissue composed of several different fiber types that are characterized on the basis of the content of myosin heavy chain type I, IIA, IIX, or IIB (Schiaffino *et al.*, 1986; Schiaffino and Reggiani, 1994). The fiber types also differ in terms of metabolism. Mitochondrial content and oxidative metabolism are high in type I muscle fibers, while type IIB muscle fibers contain low levels of oxidative enzymes and mitochondria, but have a high abundance of glycolytic proteins (Pette and Staron, 1990). Type IIA and IIX fibers are intermediate in terms of combined oxidative and glycolytic capacity. Furthermore, mitochondria from type I and type II skeletal muscle have previously been shown to differ with respect to production of reactive oxygen species. Mitochondrial free radical leak (expressed as moles of H_2O_2 produced/mole O_2 consumed), as found in the lateral portion of the gastrocnemius that is enriched in type IIB fiber content) was reported to be higher than mitochondrial free radical leak in muscles comprised mostly of type IIA fibers (medial gastrocnemius) or type I fibers (soleus) (Anderson and Neufer, 2006; Picard *et al.*, 2008). Consistent with these findings, muscles comprised primarily of type II fibers have been shown to have increased levels of mitochondrial protein oxidative damage and decreased mitochondrial function relative to muscle (soleus) enriched in type I muscle fibers (Feng *et al.*, 2008; Conley *et al.*, 2007). Furthermore, type II muscle fibers have been shown to be more susceptible to age-related changes than type I skeletal muscle (Tomonaga 1977; Larsson *et al.*, 1978; Jakobsson *et al.*, 1990; Lexell and Taylor, 1991; Fulle *et al.*, 2004). Collectively, these data suggest that mitochondrial oxidative stress and damage and mitochondrial dysfunction in type II fibers may be important factors in age-related muscle atrophy. The *TnIFastCreSod2*$^{fl/fl}$ mouse model targets deletion of MnSOD specifically in type IIB muscle fibers and is therefore particularly relevant for studying age-related changes in skeletal muscle.

Our data also strengthen the direct link between reduced MnSOD content and specific deficits in the activity of aconitase (Gardner *et al.*, 1994; Li *et al.*, 1995; Longo *et al.*, 1999; Powell and Jackson, 2003, Hinerfeld *et al.*, 2004; Lustgarten *et al.*, 2009), complex I (Longo *et al.*, 1999; Powell *et al.*, 2003; Hinerfeld *et al.*, 2004; Nojiri *et al.*, 2006; Kuwahara *et al.*, 2010), and complex II (Li *et al.*, 1995; Longo *et al.*, 1999; Powell *et al.*, 2003; Hinerfeld *et al.*, 2004; Nojiri *et al.*, 2006; Kuwahara *et al.*, 2010). Complex I supported respiration (GM) and activity were both decreased by 30%, but this amount was insufficient to adversely affect the rate of mitochondrial ATP production found in young *TnIFastCreSod2*$^{fl/fl}$ mice when compared with the corresponding value in wild type. One possible explanation involves the mitochondrial threshold effect (Mazat *et al.*, 2001), in which 35–40% of complex I must be inactivated before mitochondrial function is affected (Barrientos and Moraes, 1999). In contrast, the reduction in complex II activity found in *TnIFastCreSod2*$^{fl/fl}$ mice was sufficient to negatively affect its respective rates of mitochondrial respiration, ATP and H_2O_2 production (Lustgarten *et al.*, 2009). Succinate dehydrogenase is comprised of four nuclear encoded subunits: *SdhA*, *SdhB*, *SdhC*, and *SdhD*. SDHA is a 70 kDA protein

and contains the FAD binding site. SDHB is a 30 kDA protein and contains three iron-sulphur clusters. Both SDHA and SDHB are soluble in the mitochondrial matrix, and are anchored to the inner mitochondrial membrane by two 15 kDA transmembrane proteins, SDHC and SDHD (Hagerhall, 1997). The activity of complex II has been previously reported to be directly proportional to the content of its catalytic subunits, SDHA and SDHB (Hinerfeld *et al.*, 2004). SDHA and SDHB protein content found in type IIB enriched skeletal muscle were each significantly reduced but, no significant difference was observed for the mRNA levels of either *SdhA* or *SdhB* in *TnIFastCreSod2*$^{fl/fl}$ mice. These results are consistent with a previous study in mice lacking MnSOD in both heart and skeletal muscle (H/M-*Sod2*$^{-/-}$ mice, Nojiri *et al.*, 2006). SDHB contains three iron-sulphur clusters, leaving open the possibility that SDHB is translated, oxidized, and subsequently degraded, as it may pass through the same metabolic fate as aconitase-reversible posttranslational inactivation, release of a labile iron from the [4Fe-4S]$^{2+}$ cluster, disassembly of the [4Fe-4S]$^{2+}$ cluster, carbonylation, and, protein degradation (Bulteau *et al.*, 2003). SDHB contains one [4Fe-4S]$^{2+}$ cluster, and the [3Fe–4S]$^{+}$ cluster found in SDHB has been shown to be sensitive to oxidation (Beinert *et al.*, 1977). Here we show that purified SDH is sensitive to inactivation by superoxide, with significant time-dependent reductions in SDH activity following incubation with superoxide. Prior to our study, superoxide had been shown to be only a mild inactivator of SDH (Zhang *et al.*, 1990). Inactivation by superoxide is not a general effect as the enzymatic activity of fumarase and isocitrate dehydrogenase were not sensitive to inactivation when assayed under the same experimental conditions used to test the superoxide sensitivity of SDH. Mammalian fumarase activity was previously shown to be insensitive to superoxide-mediated inactivation (Patel *et al.*,1996), and our data validates that result.

One possible explanation for the reduction in SDHA and SDHB protein content may involve mRNA oxidation. Mitochondria isolated from type IIB enriched muscle in *TnIFastCreSod2*$^{fl/fl}$ mice respiring on complex I or complex II-linked substrate release greater than 2-fold more superoxide than mitochondria isolated from wild type mice (Lustgarten *et al.*, 2009). As a result of potentially elevated cytosolic superoxide (or H_2O_2, after conversion by CuZnSOD), an increase in *SdhA* or *SdhB* mRNA oxidation may occur, thereby resulting in a reduction in protein expression (Shan *et al.*, 2007) that may be responsible for the decrease in SDHA and/or SDHB protein content. Future studies aimed at investigating the association between a decrease in MnSOD content and the reduction in SDHA and SDHB subunit levels should investigate whether *SdhA* and *SdhA* mRNA are oxidized and are unable to be translated.

In summary, we report that mitochondrial oxidative stress and damage and mitochondrial dysfunction specifically targeted to type IIB skeletal muscle fibers over the lifespan of mice are not sufficient to accelerate age-related muscle atrophy. The fact that reductions in MnSOD activity of 50% in both young and old *Sod2*$^{+/-}$ mice (Mansouri *et al.*, 2006), greater than 80% (*present report*, young and old) and 100% in both M-*Sod2*$^{-/-}$ (Kuwahara *et al.*, 2010) and H/M-*Sod2*$^{-/-}$ mice (Nojiri *et al.*, 2006) all result in an increase in mitochondrial oxidative stress and compromised mitochondrial function without affecting skeletal muscle mass strongly argue that mitochondrial oxidative stress and mitochondrial dysfunction are not involved in the maintenance of muscle mass in either young or old mice.

Experimental Procedures

Animals

Young (6–8 months) and old female (27–34 months) wild type and *TnIFastCreSod2*$^{fl/fl}$ mice (described previously in Lustgarten *et al.* 2009) were used for all experiments, with the exception of the assay of F_2-isoprostanes, in which 21-month-old wild type and

TnIFastCreSod2$^{fl/fl}$ male mice were used. All procedures involving the mice were approved by the Subcommittee for Animal Studies at the Audie L. Murphy Veterans Administration Hospital and the University of Texas Health Science Center at San Antonio.

Measurement of mitochondrial function

Because the TnI promoter is expressed predominantly in type IIB muscle fibers (Hallauer and Hastings, 2002), we used portions of mouse hindlimb muscle that are predominantly type II in composition (the white portion of the quadriceps and the gastrocnemius) for isolation of mitochondria for our experiments and refer to this as type IIB enriched muscle throughout the manuscript. Burkholder et *al.*, (1994) reported the type IIB fiber composition of the gastrocnemius and quadricep group to range from 55–70%. Red portions of both muscles were removed to maximize type IIB fiber content in our muscle preparations. We have previously shown that MnSOD activity is reduced by greater than 80% in this combined muscle preparation (Lustgarten *et al.* 2009).

Mitochondria were isolated based on the method of King *et al.*, (2007) and as described by Lustgarten *et al.*, (2009). Mitochondrial respiration was measured using the method of Hynes *et al.*, (2006). This assay is based on measuring the fluorescence of the oxygen-sensing probe (A65N-1, Axxora, San Diego, CA) which is quenched in the presence of oxygen. The quenching of the signal declines as oxygen is consumed, and the corresponding increase in fluorescence is directly proportional to the rate of mitochondrial oxygen consumption. 40–80 μg of mitochondria isolated from type IIB enriched muscle from wild type and *TnIFastCreSod2$^{fl/fl}$* mice was resuspended in 150 μL of respiration buffer containing 125 mM KCl, 10 mM MOPS, 5 mM $MgCl_2$, 2 mM K_2HPO_4, pH 7.44, with substrates: GM (12.5 mM glutamate, 12.5 mM malate) or SR (25 mM succinate, 0.5 μM rotenone) and A65N-1 probe, 67 nM. 100 μL of mineral oil was used to seal each well from atmospheric oxygen. Measurements were performed in a Spectramax 384 spectraphotometer (Molecular Devices, U.S.A.).

The rate of mitochondrial ATP production was measured in isolated muscle mitochondria as we have previously described (Lustgarten *et al.*, 2009). The respiratory substrates GM (2.5 mM), SR (5 mM succinate, 0.5 μM rotenone), α-ketoglutarate (2.5 mM) and α-glycerol phosphate (2.5 mM) were added as indicated. Mitochondrial electron transport respiratory complex activity (ETC, complexes I-IV) was measured using spectrophotometric assays. Mitochondria isolated from type IIB enriched skeletal muscle isolated from wild type and *TnIFastCreSod2$^{fl/fl}$* mice were solubilized in buffer containing 750 mM 6-aminocaproic acid (hexanoic acid), 50 mM Bis Tris and 1% L-dodecyl maltoside, pH 7.0. The samples were then placed on a rotator for 1 hour at 4°C, followed by centrifugation at 100,000*xg* for 15 minutes. The supernatant was then assayed for protein content and resuspended in respiration buffer (250 mM sucrose, 10 mM KH_2PO_4, 10 mM Tris, 1 mM EGTA, pH 7.4). Activity measurements for complexes I, II and IV were performed on a Spectramax 384 Spectraphotometer (Molecular Devices, U.S.A). Complex III activity was measured via a Beckman spectrophotometer. For complex I, 3.3 μg of mitochondrial protein was added to 150 μL of respiration buffer containing 0.1 mM NADH, 0.05 mM decylubiquinone and 0.05 mM dichloroindophenol (DCIP). Complex I activity was determined by monitoring the oxidation of NADH at 340 nm, at 30°C (Birch-Machin and Turnbull, 2001). A separate sample was measured in parallel with the addition of 640 nM rotenone. Subtraction of this data is indicative of rotenone-sensitive complex I activity. Complex I data was expressed as Units of NADH oxidized/min/mg protein. For complex II, 3.3 μg of mitochondrial protein was added to 150 μL of respiration buffer containing 20 mM succinate, 50 uM ubiquinone, 2 mM KCN and 50 μM DCIP. Complex II activity was measured via the succinate-dependent reduction of DCIP, as measured at 600 nm, at 30°C (Boveris and Cadenas, 1975). A separate sample containing 3 mM malonate was measured in parallel. Subtraction of this data is

indicative of malonate-sensitive complex II activity. Complex II data are expressed as Units DCIP reduced/min/mg protein. For complex III, 3.3 μg of mitochondrial protein was added to 1 mL of buffer containing 100 μM (Fe^{3+}) cytochrome c, 100 μM decylubiquinol and 2 mM KCN. Complex III activity was measured by following the reduction of cytochrome c at 550 nm, at 25°C. Complex III data was expressed as Units of cytochrome c reduced/min/mg protein. Complex IV activity was measured by addition of 1.65 μg of mitochondrial protein to 150 μL of respiration buffer containing 90 μM (Fe^{2+}) cytochrome c. Complex IV activity was determined by following the oxidation of cytochrome c at 550 nm, at 30°C. Complex IV data are expressed as Units of cytochrome c oxidized/min/mg protein.

Histology

Cryostat sections of gastrocnemius muscles isolated from young female wild type and *TnIFastCreSod2* $^{fl/fl}$ mice were used for the histologic analysis of complex II and complex IV activity and were prepared as previously described by Lustgarten *et al.*, (2009). Complex II activity was assayed by incubating sections for 1 hour at 37°C in a buffer containing 20 uM $NaPO_4$, 40 μM succinate, 0.1% NBT, pH 7.4 (Nachlas *et al.*, 1957). Sections were then rinsed in saline, followed by fixation in formalin-saline (10% neutral buffered formalin containing 10 mM NaCl) for 10 minutes. Sections were placed in 15% ethanol for 15 minutes, followed by addition of mounting media. A dark blue formazan color is indicative of muscle fibers rich in complex II activity. Complex IV activity was assayed by incubating sections for 1 hour at room temperature in buffer consisting of 200 mM sucrose, 20 mM $NaPO_4$, 1.3 mM 3, 3′ diaminobenzidine tetrahydrochloride (DAB), 80 μM cytochrome c, 8 nM catalase, pH 7.6. Sections were then washed three times with double distilled water, followed by muscle section dehydration in ascending alcohols (50%, 70%, 80%, 95% × 2 washes, 100% × 2 washes). Sections were cleared with two additions of xylene. Following the addition of mounting media, sections were visualized for dark brown staining, which is indicative of muscle fibers with high complex IV activity (Seligman, 1972).

Measurement of SDH mRNA

SdhA and *SdhB* mRNA were isolated from type IIB enriched skeletal muscle and the content was measured using quantitative real time PCR as described by Dahia *et al.*, (2005). For determination of *SdhA* mRNA content, GTGCGGATTGATGAGTACGATT and CACATGCATGAGCTATTATACATAA were used as the forward and reverse primers, respectively. *SdhB* mRNA content was measured with use of CGACTCCAGAGACGACTTCAC and GCTCGCTTCTCCTTGTAGGTC as the forward and reverse primers, respectively.

Western Blotting

Homogenates of type IIB enriched or type I enriched (soleus) skeletal muscle isolated from *TnIFastCreSod2*$^{fl/fl}$ and wild type mice were prepared in assay buffer containing 50 mM Tris-HCl buffer with 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate and 1*x* protease inhibitor. Equivalent amounts of protein (40–80 μg) for each sample were resolved in 4–20% Tris-HCl SDS-PAGE gels in triplicate. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane. The membrane was then incubated in Tris-buffered saline, pH 7.4, with 0.05% Tween 20 (TBS-T) containing 10% nonfat milk for 1 hour at room temperature. The blots were then reacted with antibodies specific for aconitase (1:2000, a kind gift from Luke Szweda, Oklahoma Medical Research Foundation, Oklahoma, U.S.A.), SDHA (1:1000, Invitrogen, U.S.A.) and SDHB (1:500, Invitrogen, U.S.A.) at 4°C overnight. After washing with TBS-T, the blots were incubated with either goat anti-rabbit IgG horseradish peroxidase or goat anti-mouse IgG-horseradish peroxidase (1:1000, Sigma) for 2 hours at room temperature. The blots were washed five times with

TBS-T and the bands corresponding to each respective protein was visualized using chemiluminescent detection reagents obtained from Amersham Biosciences.

Citric acid cycle enzymatic activity

Citric acid cycle enzymatic activity was measured in mitochondria isolated from type IIB enriched-skeletal muscle from wild type and *TnIFastCreSod2*$^{fl/fl}$ mice. To obtain soluble mitochondrial proteins, mitochondria were resuspended in 30 mM potassium phosphate buffer containing 0.2% Triton-X-100, pH 7.4, followed by rotation for 45 minutes at 4°C and centrifugation at 16100*xg*. The activity of citrate synthase (1 μg), isocitrate dehydrogenase (13 μg), fumarase (30 μg) and malate dehydrogenase (0.1) was measured by addition of mitochondrial extract (with the amount of protein added in parenthesis) to 200 μL of enzyme activity buffer as described by Robinson *et al.*, (1987). All enzyme activity assays were conducted at 30°C.

Identification of proteins sensitive to superoxide

Purified *E. Coli* succinate dehydrogenase (SDH, a kind gift from Dr. Gary Ceccini, UCSF), fumarase (Sigma-Aldrich, U.S.A.) and isocitrate dehydrogenase (Sigma-Aldrich, U.S.A.) were used to determine their potential sensitivity to inactivation in the presence of potassium superoxide. *E. Coli* SDH is structurally and functionally homologous to mammalian SDH (Cecchini *et al.*, 2002). Potassium superoxide solution was prepared by adding 0.5 g of KO_2 to 1.5 mL anhydrous dimethyl sulfoxide (DMSO). This solution was vortexed for 5 minutes, followed by centrifugation at 500*xg* for 5 minutes. The supernatant containing soluble KO_2 was removed. Catalase was added (1/100) to degrade any hydrogen peroxide that had formed from the spontaneous dismutation of superoxide. Electron paramagnetic resonance (EPR) was used to quantify the amount of superoxide present in the KO_2 solution. The KO_2 stock was then further diluted in DMSO (1/5) to obtain an EPR spectrum intermediate in magnitude to that produced by mitochondria isolated from both wild type and *TnIFastCreSod2*$^{fl/fl}$ mice (Lustgarten *et al.*, 2009). Non-diluted KO_2 produces an EPR signal that is approximately 10–12 and 5–6 fold higher than mitochondria respiring on SR and isolated from wild type and *TnIFastCreSod2*$^{fl/fl}$, respectively (*data not shown*). Addition of CuZnSOD (1 U/μL) eliminated the observed EPR spectra (Lustgarten *et al.*, 2009), thereby indicating the specificity of the EPR-derived signal for detecting superoxide. Individual enzymes were assayed for their sensitivity to inactivation in the presence of superoxide via room temperature incubation. For example, 10 μg of purified SDH was added to either 1 μL of diluted KO_2 or 1 μL of DMSO in a final volume of 10 μL. For measurement of SDH activity, 200 mM succinate, 4 mM ubiquinone and 3 mM DCIP were added to a reaction buffer consisting of 250 mM sucrose, 10 mM KH_2PO_4, 10 mM Tris, 1 mM EGTA, 0.4 U/μL CuZnSOD, 7.5U/μL catalase, pH 7.4. CuZnSOD and catalase were added to the reaction buffer to degrade any superoxide (and potentially, H_2O_2) that remained after incubation with SDH. At the indicated time points (0, 10, 20, 40 minutes), 0.6 μL of succinate dehydrogenase incubated with either KO_2 or DMSO was removed and added to 150 μL of reaction buffer. SDH activity was then measured based the method of Boveris and Cadenas (1975).

To test the sensitivity of other mitochondrial matrix-localized proteins to inactivation by superoxide, we measured the enzymatic activity of fumarase and isocitrate dehrdrogenase. Three μg of fumarase was incubated with 4 μL of diluted KO_2 stock or 4 μL of DMSO in 50 μL of reaction buffer. At the indicated time points (0, 10, 20, 40 minutes) 10 μL of superoxide or DMSO incubated-fumarase was added to150 μL of reaction buffer containing 100 mM malate, 0.4 U/μL CuZnSOD and 7.5 U/μL catalase, pH 7.4. Fumarase activity was measured based on the method of Robinson *et al.*, (1987). Similarly, 55 μg of isocitrate dehydrogenase was incubated with 3.75 μL of diluted KO_2 or 3.75 μL of DMSO in 50 μL of

reaction buffer. At the indicated time points (0, 10, 20, 40 minutes), 10 μL of superoxide or DMSO-incubated isocitrate dehydrogenase was added to 150 uL of reaction buffer containing 50 mM Tris pH 7.4, 1 mM $MnCl_2$, 0.5 mM EDTA, 0.25 mM NADP+, 1.6 mM isocitrate, 0.4 U/μL CuZnSOD and 7.5U/μL catalase, pH 7.4. Isocitrate dehydrogenase activity was then measured based on the method of Robinson *et al.*, (1987).

Measurement of oxidative stress status during aging in TnIFastCreSod2$^{fl/fl}$ mice

Measurement of aconitase activity is a sensitive indicator of mitochondrial matrix superoxide content (Gardner and Fridovich, 1991; Gardner *et al.*, 1994, 1995, Gardner 2002). Aconitase activity was measured in homogenates of type IIB enriched skeletal muscle isolated from old female *TnIFastCreSod2$^{fl/fl}$* and wild type mice, based on the method of Gardner *et al.*, (1994) and as described by Lustgarten *et al.*, (2009). Mitochondrial superoxide release was measured via electron paramagnetic resonance (EPR) with use of the spin trap, 5-diisopropoxyphosphoryl-5-methyl-1-pyrroline-N-oxide, (DIPPMPO; Chalier and Tordo, 2002), as described by Lustgarten *et al.*, (2009). Levels of F_2-isoprostanes in tibialis anterior (TA) muscles isolated from middle aged (21 months) mice were determined as described by Lustgarten *et al.*, (2009). TA muscle has been shown to contain 35% type IIB and 65% IIA fibers (Burkholder *et al.*, 1994)

Statistics

Unpaired student's t test was used for all analyses, unless otherwise indicated.

Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Co., unless otherwise indicated.

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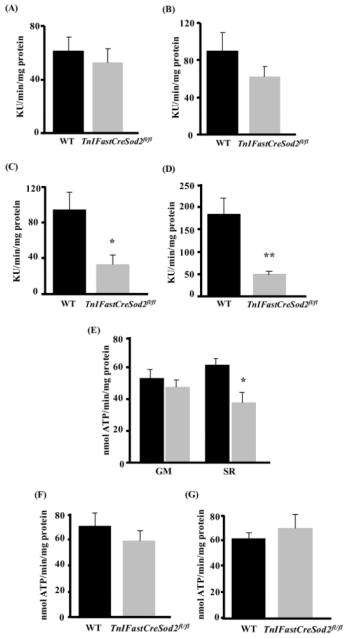

Figure 1. Mitochondrial function in young *TnIFastCreSod2$^{fl/fl}$* and wild type mice

A) Basal and maximal mitochondrial oxygen consumption were measured in mitochondrial isolated from type IIB enriched muscle fibers isolated from young (6–8 month) *TnIFastCreSod2$^{fl/fl}$* and wild type mice using the oxygen sensing probe, A65N-1, as described in Experimental Procedures. Basal respiration (A, C; n=5) was measured in the absence of exogenously added ADP with glutamate plus malate (GM, A) and succinate plus rotenone (SR, C) used as substrates. Maximal respiration was measured in the presence of exogenously added ADP with GM (B, n = 9) and SR (D, n = 10) used as substrates. Data are expressed as Kilo Units (KU, 10^3)/min/mg protein. *denotes a significant difference ($p < 0.05$) from wild type values. **denotes a significant difference ($p < 0.01$) from wild type values. Values represent means ± SEM. E–G) Rate of mitochondrial ATP production in mitochondrial isolated from type IIB enriched hindlimb skeletal muscle of 6–8 month *TnIFastCreSod2$^{fl/fl}$* (*grey bars*) and wild type (*black bars*). ATP production was measured using a luciferase based assay as described in Experimental Procedures. GM and SR (E) were used as substrate. α-ketoglutarate (F) and α-glycerolphosphate (G) were used as substrates. Values represent means ± SEM for five samples.

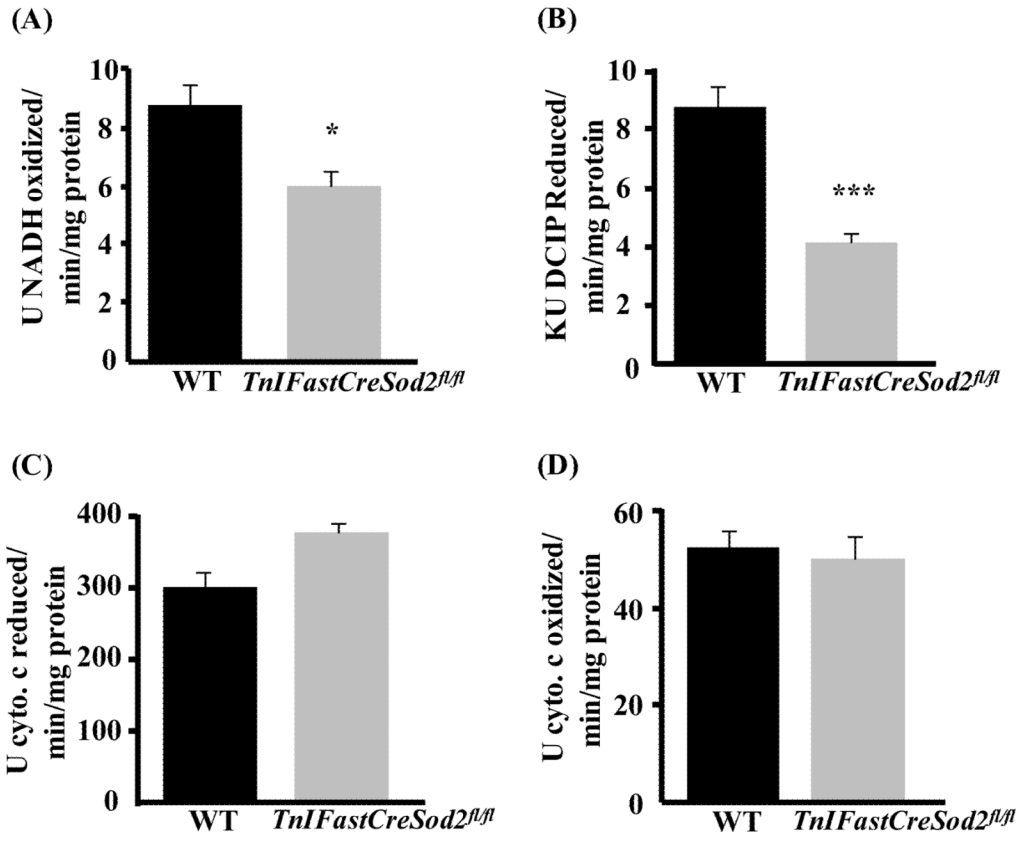

Figure 2. Biochemical measurement of ETC complex activity in young *TnIFastCreSod2*$^{fl/fl}$ and wild type mice

Electron transport chain respiratory complex activity was measured spectrophotometrically as described in Experimental Procedures. A–D) ETC activity in mitochondria isolated from type IIB enriched skeletal muscle from young (6–8 month) *TnIFastCreSod2*$^{fl/fl}$ and wild type mice for A) complex I, B) complex II, C) complex III, D) complex IV. Values represent means ± SEM for 5 samples. *denotes a significant difference ($p < 0.05$) from wild type values. ***denotes a significant difference ($p < 0.001$) from wild type values.

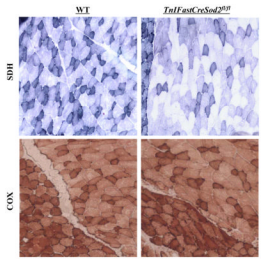

Figure 3. Histological measurement of complex II and complex IV activity in young *TnIFastCreSod2$^{fl/fl}$* and wild type mice

Gastrocnemius (containing the attached soleus) was frozen in liquid nitrogen-cooled isopentane and sectioned using a cryostat. Muscle sections were incubated with substrates specific for complex II (*Top)* and complex IV (*Bottom*), as described in Experimental Procedures. Each image is a representative section of four independent samples for both wild type (*left*) and *TnIFastCreSod2$^{fl/fl}$* (*right*).

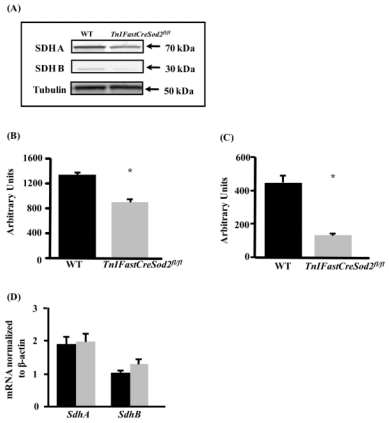

Figure 4. *SdhA* and *SdhB* mRNA and protein content in young *TnIFastCreSod2*$^{fl/fl}$ and wild type mice

SDHA and SDHB protein content found in type IIB enriched skeletal muscle (A) in young (6–8 month) *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. Tubulin was used as the loading control. Quantification of SDHA and SDHB is shown in Figure 4B and 4C, respectively. Each image is representative ofseven samples for both *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. mRNA expression of *SdhA* and *SdhB* in type IIB enriched skeletal muscle (D) in *TnIFastCreSod2*$^{fl/fl}$ (*grey bars*) and wild type (*black bars*) mice was measured as described in Experimental Procedures. Values represent means ± SEM for five samples.

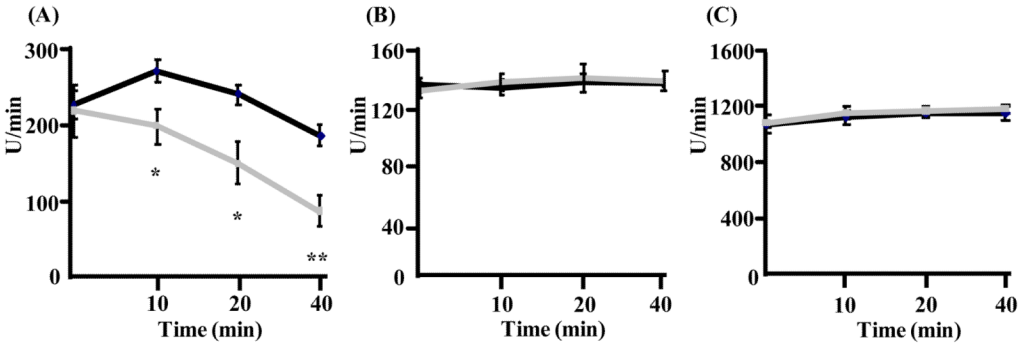

Figure 5. Sensitivity of SDH, isocitrate dehydrogenase and fumarase to inactivation after incubation with superoxide for 0, 10, 20, and 40 minutes

SDH (A), isocitrate dehydrogenase (B) and fumarase (C) were incubated for 0, 10, 20 and 40 minutes with an amount of potassium superoxide intermediate to that produced by actively respiring mitochondria isolated from type IIB enriched skeletal muscle in young (6–8 month) *TnIFastCreSod2*$^{fl/fl}$ and wild type mice. Superoxide was measured via use of DIPPMPO and EPR. Addition of CuZnSOD eliminated the EPR-derived signal, indicating that superoxide was responsible for the enzymatic inactivation of SDH (Lustgarten *et al.*, 2009). Values represent means ± SEM for five samples for SDH, four samples each for isocitrate dehydrogenase and fumarase. *denotes a significant difference ($p < 0.05$) from wild type values. **denotes a significant difference ($p < 0.01$) from wild type values.

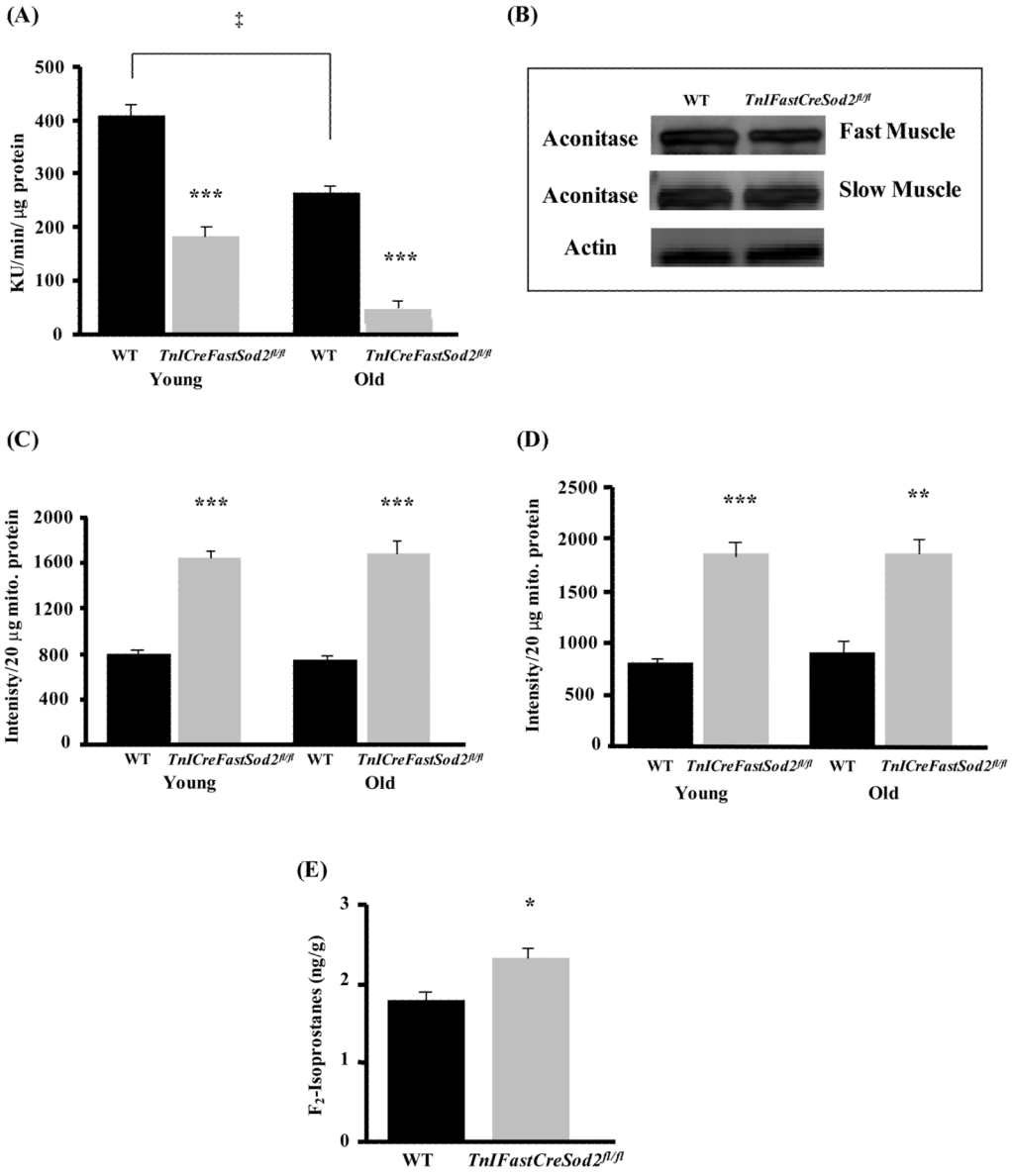

Figure 6. Oxidative stress in old *TnIFastCreSod2$^{fl/fl}$* and wild type mice

Aconitase activity (A) was measured in homogenates of type IIB enriched skeletal muscle from old (27–34 months) wild type and *TnIFastCreSod2$^{fl/fl}$* mice. Values represent means ± SEM for five samples. ***denotes a significant difference ($p < 0.001$) from wild type values. ‡Significantly ($p < .05$) different as a function of age. Aconitase protein content (B) in type IIB enriched skeletal muscle and oxidative skeletal muscle (soleus) isolated from old (27–34 months) *TnIFastCreSod2$^{fl/fl}$* and wild type mice. Each image is representative of three samples each for both old wild type and *TnIFastCreSod2$^{fl/fl}$* mice. Actin was used as a loading control in type II enriched muscle. Mitochondrial superoxide release was measured in mitochondria that respired on GM (C) or SR (D), as isolated from young (6–8 months) and old (27–34 months) wild type (*black bars*) and *TnIFastCreSod2$^{fl/fl}$* (*grey bars*). Values represent means ± SEM for five samples. ***denotes a significant difference ($p < 0.001$) from wild type values. E) Lipid peroxidation (F_2-isoprostanes) in tibialis anterior muscles isolated from middle aged (21 months) *TnIFastCreSod2$^{fl/fl}$* and wild type mice, measured as described in Experimental Procedures, and, expressed as nanograms per gram of tissue.

Values represent means ± SEM for three samples for wild type and four samples for *TnIFastCreSod2*$^{fl/fl}$ mice. *denotes a significant difference ($p < 0.05$) from wild type values.

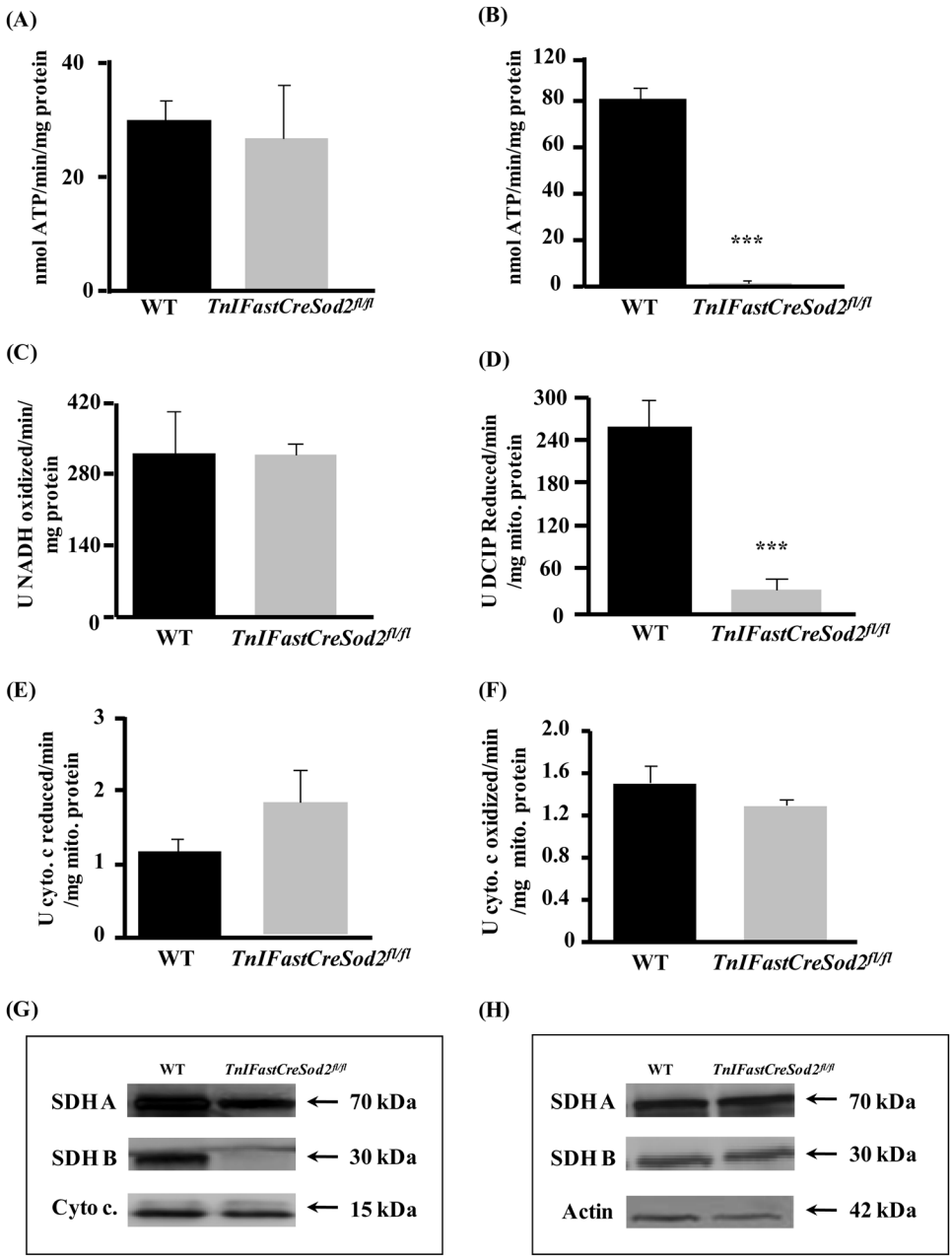

Figure 7. Deficits in complex II-linked ATP production, complex II activity and protein content in old *TnIFastCreSod2$^{fl/fl}$* mice

The rate of mitochondrial ATP in old (27–34 months) wild type and *TnIFastCreSod2$^{fl/fl}$* mice, with GM (A) and SR (B) used as respiratory substrates. Values represent means ± SEM for five samples. ***denotes a significant difference ($p < 0.001$) from wild type values. Biochemical measurement of ETC complex activity (complex I, (C); complex II, (D); complex III, (E); complex IV, (F) in mitochondria isolated from type IIB enriched skeletal muscle in old *TnIFastCreSod2$^{fl/fl}$* and wild type mice, measured as described in Experimental Procedures. Values represent means ± SEM for four samples. ***denotes a significant difference ($p < 0.001$) from wild type values. SDHA and SDHB protein content in homogenates of type IIB enriched skeletal muscle (G) and oxidative (H) skeletal muscles isolated from old *TnIFastCreSod2$^{fl/fl}$* and wild type mice. Each image is representative of

three samples for both wild type and *TnIFastCreSod2*$^{fl/fl}$ mice. Cytochrome c and actin were used as loading controls for type II enriched skeletal muscle and in type I enriched muscle, respectively.

Table I

Citric acid cycle (TCA) enzymatic activity in type IIB enriched skeletal muscle isolated from young (6–8 month) *TnIFastCreSod2$^{fl/fl}$* and wild type mice

TCA enzymatic activity was measured as described in Experimental Procedures. For citrate synthase, aconitase, isocitrate dehydrogenase, fumarase, and malate dehydrogenase (n = 4). For succinate dehydrogenase (n = 5). Data are expressed as Kilo Units (KU, 10^3)/min/mg protein for citrate synthase, succinate dehydrogenase and fumarase. Data are expressed as Mega Units (MU, 10^6)/min/mg protein for aconitase, isocitrate dehydrogenase and malate dehydrogenase.

	Citrate Synthase	Aconitase	Isocitrate Dehydrogenase	Succinate Dehydrogenase	Fumarase	Malate Dehydrogenase
WT	12.8 ± 0.1	0.41 ± 0.02	6.7 ± 0.4	8.7 ± 0.7	12.3 ± 0.3	3.8 ± 0.5
TnIFastCreSod2$^{fl/fl}$	11.8 ± 0.9	0.18 ± 0.02*	6.1 ± 0.5	4.1 ± 0.4*	12.6 ± 0.2	3.7 ± 0.3

Values represent means ± SEM.

*denotes a significant difference ($p < 0.05$) from wild type values.

Table II

Phenotypic data in young and old *TnIFastCreSod2$^{fl/fl}$* and wild type mice

Shown are values for body mass, percent lean mass or body fat, and muscle mass normalized to body mass for soleus and gastrocnemius muscle during aging in young female (6–8 months, n = 5) and old (27–34 months, n = 5) wild type and *TnIFastCreSod2$^{fl/fl}$* mice. Quantitative magnetic resonance imaging (qMRI) was used for the determination of the percentages of body fat and lean mass based on Taicher *et al*., (2003), Tinsley *et al.*, (2004) and as described by Lustgarten *et al*., (2009).

		Body Mass (g)	Lean Mass (%)	Body Fat (%)	Sol./BW (mg/g)	Gast./BW (mg/g)
Young	**WT**	21.3 ± 0.3	83.2 ± 0.6	16.5 ± 0.6	0.32 ± 0.01	5.9 ± 0.1
	TnIFastCreSod2$^{fl/fl}$	20.9 ± 0.3	81.7 ± 0.6	17.8 ± 0.6	0.33 ± 0.02	5.7 ± 0.1
Old	**WT**	23.4 ± 0.8[a]	78.2 ± 2.1[a]	21.2 ± 2.1[a]	0.34 ± 0.02	4.1 ± 0.2[a]
	TnIFastCreSod2$^{fl/fl}$	25.7 ± 1.6[a]	76.1 ± 1.9[a]	23.4 ± 1.9[a]	0.30 ± 0.02	4.4 ± 0.2[a]

Values represent means ± SEM.

[a] denotes a significant difference ($p < 0.05$) as a function of age.