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# Protective effect of myostatin gene deletion on aging-related muscle metabolic decline

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ABSTRACT

While myostatin gene deletion is a promising therapy to fight muscle loss during aging, this approach induces also skeletal muscle metabolic changes such as mitochondrial deficits, redox alteration and increased fatigability. In the present study, we evaluated the effects of aging on these features in aged wild-type (WT) and mstn knock-out (KO) mice. Moreover, to determine whether an enriched-antioxidant diet may be useful to prevent age-related disorders, we orally administered to the two genotypes a melon concentrate rich in superoxide dismutase for 12 weeks. We reported that mitochondrial functional abnormalities persisted (decreased state 3 and 4 of respiration; $p < 0.05$) in skeletal muscle from aged KO mice; however, differences with WT mice were attenuated at old age in line with reduced difference on running endurance between the two genotypes. Interestingly, we showed an increase in glutathione levels, associated with lower lipid peroxidation levels in KO muscle. Enriched antioxidant diet reduced the aging-related negative effects on maximal aerobic velocity and running limit time ($p < 0.05$) in both groups, with systemic adaptations on body weight. The redox status and the hypertrophic phenotype appeared to be beneficial to KO mice, mitigating the effect of aging on the skeletal muscle metabolic remodeling.



## 1. Introduction

Skeletal muscle represents the largest organ of human body. Its main functions are to provide movement and autonomy and to balance posture as well as to regulate the body temperature. Loss of skeletal muscle occurs as a consequence of several chronic diseases (cachexia) as well as normal aging (sarcopenia), and may play a role in reduced physical performance, falls, disability, and mortality for an increasing number of elderly patients (Fielding et al., 2011). Consequently, numerous potential therapeutic approaches are being considered and developed. One of the most promising approaches is inhibition of the myostatin signalling (McPherron et al., 1977; Matsakas, 2014; Rodriguez et al., 2014).

Myostatin (mstn), a member of the transforming growth factors superfamily, is a highly conserved negative regulator of skeletal muscle mass (McPherron et al., 1977). The induced or natural conditions leading to myostatin inactivation or inhibition result in an increased muscle mass and are considered as promising therapeutic approaches to prevent muscle wasting associated with numerous disorders such as myopathies (Hulmi et al., 2013; Amthor and Hoogaars, 2012), cancer (Busquets et al., 2012; Gallot et al., 2014), or aging (Morissette et al., 2009; Collins-Hooper et al., 2014; Mendias et al., 2015). In addition to its role in skeletal muscle growth, myostatin has recently been reported to be significantly involved in the regulation of muscle metabolism. Indeed, early studies provided evidence at the cellular level that deletion of *myostatin* in glycolytic skeletal muscles led to loss of oxidative properties, with impaired activity of oxidative metabolism enzymes, a reduced capillary density, mitochondrial DNA depletion, and an uncoupled respiration in the intermyofibrillar mitochondria (Amthor et al., 2007; Lipina et al., 2010; Ploquin et al., 2012). This disturbance in oxidative metabolism and mitochondrial deficits is also associated with an alteration in redox homeostasis (Ploquin et al., 2012; Sriram et al., 2011) and increased muscle fatigability (Giannesini et al., 2013; Relizani et al., 2014; Mouisel et al., 2014). Notably, mstn-deficient fast-twitch skeletal muscles exhibit a significant decrease in lipid peroxidation levels together with a significant upregulation of the antioxidant glutathione system. By contrast, mitochondrial superoxide dismutase (MnSOD) and catalase activities are compromised with a reduction of up to 80% compared to control muscles (Ploquin et al., 2012). The mitochondrial deficit and the redox homeostasis alteration in skeletal muscle raise a number of questions. First, does *mstn* deletion alter mitochondrial yield and redox signaling in other organs with high oxidative potential such as the liver?

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Indeed, available data have not explored whether these metabolic features were exclusive to skeletal muscles. Second, are the metabolic features described in young mstn-deficient mice attenuated or worsened with aging? Numerous papers have assigned mitochondrial function and redox status as the primary (initially) or secondary (new vision) causes of age-related muscle disorders (Derbré et al., 2014). At subcellular level, aged muscle fibers display an increased level of mitochondrial abnormalities and susceptibility to apoptosis (Chabi et al., 2008). Aged mammalian and human populations exhibited higher muscle oxidative damage, as indicated by reduced glutathione levels and increased protein and DNA oxidation as well as lipid peroxidation (Marzani et al., 2005; Pansarasa et al., 1999). In this context, the consequences of *mstn* deletion on muscle redox status and mitochondrial metabolism in aged mice remain to be investigated. Despite the deficit in oxidative metabolism, the global reduced redox state in mstn-deficient skeletal muscle could be beneficial to counteract age-related oxidative stress. By contrast, the fast-twitch muscle phenotype observed in young mice with natural or constitutive deletion of *mstn* (Girgenrath et al., 2005) associated to the strong decrease in MnSOD activity reported in glycolytic muscles could be thus a disadvantage for "healthy aging." Indeed, decrease or lack of adaptation in antioxidant activities, such as MnSOD and GPx, has been reported in glycolytic muscles (Derbré et al., 2014). Finally, it would be interesting to study the effect of dietary antioxidant-enriched supplementation in aged mice with and without *mstn* deletion. An original way could be to supply antioxidant enzymes to the mice that have long-lasting effects due to their lower rate of exhaustion compared to mere metabolites, such as SODB, a melon concentrate particularly rich in SOD with reported beneficial antioxidant effects in several models with increased oxidative stress (Carillon et al., 2013).

Thus, the aim of this study is to identify the effect of aging on the regulation of mitochondrial function and redox status by constitutive *mstn* deletion, in glycolytic skeletal muscles and liver, and to determine whether an antioxidant diet may be useful to prevent age-related disorders. Here we demonstrated that mitochondrial function and redox signaling are specifically altered in skeletal muscle compared to liver in mstn-deficient mice. Beyond muscle hypertrophy, we showed that a reduced redox status persists in aged mstn KO muscle. Moreover, the metabolic differences between WT and KO mice tended to diminish with age, in particular, mitochondrial function. In this direction, the difference in running endurance between both groups of aged mice is also attenuated. In both genotypes, supplementation by an enriched antioxidant diet reduced the negative aging effects on running outcomes, associated with systemic adaptations as seen on the body weight measurements. The redox status and the hypertrophic phenotype appeared as beneficial adaptations in KO mice because it has attenuated the effect of aging on the skeletal muscle metabolic remodeling.

## 2. Experimental procedures

### 2.1. Animals

Twenty-month-old male mstn KO (aged KO, $n = 16$) and wild-type male mice (aged WT, $n = 17$) and 2-month-old male mstn KO (young KO, $n = 10$) and wild-type male mice (young WT, $n = 10$) were included in the study. Male mstn KO mice used in this study have been described previously (Grobet et al., 2003). WT and mstn KO mice were produced from homozygous matings, and genotypewas determined by PCR analysis of tail DNA.

Mice were fed ad libitum and kept under a 12:12-h light–dark cycle. The experimental protocols of this study were handled in strict accordance with European directives (86/609/CEE) and approved by the Ethical Committee of Region Languedoc Roussillon.

### 2.2. Antioxidant supplementation

SOD by Bionov (SODB, Avignon, France) is a melon (not Genetic Modified Organism) concentrate, particularly rich in SOD, resulting from a patented extraction process. For nutraceutical applications, SODB is coated with palm oil, by spray drying method, in order to preserve SOD activity from the digestive enzymes secreted above the small intestine. In this study, SODB contains 14 U SOD/mg powder measured according to Zhou and Prognon (2006). Aged WT and mstn KO mice received the SOD-melon concentrate SODB, mixed with food, at the daily dose of 40 U SOD for 3 months (aged WT + SODB; aged KO + SODB, $n = 8$ in each group).

### 2.3. Aerobic exercise tolerance and endurance capacity

Young and aged mice were submitted to two running tests on a treadmill. Maximal aerobic velocity (MAV) was determined following a running test, where the speed was gradually increased by 2 m/min from 10 m/min until exhaustion. Exhaustion was defined when the mice were no longer able to maintain their normal running position and/or after 5 consecutive seconds in contact with the shock grid (≤0.2 mA) at the rear of the treadmill. Endurance capacity was determined via a submaximal running test where the speed starts at 10 m/min for the first 2 min and was set to 70% of MAV until exhaustion. The endurance limit time was recorded. Mice were killed 48 h after the last running test.

Same running tests were performed in blinded manner in aged mice with (aged WT + SODB; aged KO + SODB) and without (aged WT + placebo; aged KO + placebo) the antioxidant supplementation diet, before (at 20 months) and after (at 23 months) the nutritional treatment.

### 2.4. Sampling

At the end of the dietary supplementation, mice were weighed and euthanized by cervical dislocation. Liver, quadriceps, gastrocnemius, tibialis anterior (TA), and extensor digitorum longus (EDL) were removed, weighed, and either immediately placed into ice-cold buffer (100 mM KCl, 5 mM $MgSO_4$, 5 mM EDTA, 50 mM Tris–HCl, pH 7.4) for mitochondrial isolation, or frozen in liquid nitrogen, and stored at −80 °C for enzymatic and protein analysis.

### 2.5. Mitochondrial isolation and respiration

Mixed muscles (quadriceps, gastrocnemius, and tibialis anterior) were freed from connective tissues, briefly minced, and homogenized with a Potter–Elvehjem homogenizer. Subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria were fractionated by differential centrifugation as described previously (Cogswell et al., 1993). Mitochondria were resuspended in 100 mM KCl, 10 mM MOPS, pH 7.4. Mitochondrial protein content was determined using the Bradford assay, and the yield was expressed as mg of mitochondrial proteins per gram of muscle wet weight. SS and IMF mitochondrial oxygen consumption was measured using the high-resolution Oxygraph-2 k (OROBOROS Instruments, Innsbruck, Austria) as previously described (Ploquin et al., 2012). Resting rate (state 4) was evaluated in the presence of 2.5 mM malate, 5 mM glutamate, and 5 mM succinate; ADP-stimulated rate (state 3) was determined after addition of 0.5 mM ADP. The respiratory control ratio (RCR) was set as the ratio of oxygen consumption at state 3 over oxygen consumption at state 4.

### 2.6. Mitochondrial respiratory complexes and citrate synthase activities in tissues

Mitochondrial activities were measured in gastrocnemius, and liver. Complex I activity was measured according to Cogswell et al.