Lack of myostatin impairs mechanical performance and ATP cost of contraction in exercising mouse gastrocnemius muscle in vivo

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Giannesini B, Vilmé C, Amthor H, Bernard M, Bendahan D. Lack of myostatin impairs mechanical performance and ATP cost of contraction in exercising mouse gastrocnemius muscle in vivo. Am J Physiol Endocrinol Metab 305: E33–E40, 2013. First published April 30, 2013; doi:10.1152/ajpendo.00651.2012.—Although it is well established that the lack of myostatin (Mstn) promotes skeletal muscle hypertrophy, the corresponding changes regarding force generation have been studied mainly in vitro and remain conflicting. Furthermore, the metabolic underpinnings of these changes are very poorly documented. To clarify this issue, we have investigated strictly noninvasively in vivo the impact of the lack of Mstn on gastrocnemius muscle function and energetics in Mstn-targeted knockout (Mstn−/−) mice using 1H-magnetic resonance (MR) imaging and 31P-MR spectroscopy during maximal repeated isometric contractions induced by transcutaneous electrostimulation. In Mstn−/− animals, although body weight, gastrocnemius muscle volume, and absolute force were larger (+38, +118, and +34%, respectively) compared with wild-type (Mstn+/+) mice, specific force (calculated from MR imaging measurements) was significantly lower (−36%), and resistance to fatigue was decreased. Besides, Mstn deficiency did not affect phosphorylated compound concentrations and intracellular pH at rest but caused a large increase in ATP cost of contraction (up to +206% compared with Mstn+/+) throughout the stimulation period. Further, Mstn deficiency limits the shift toward oxidative metabolism during muscle activity despite the fact that oxidative ATP synthesis capacity was not altered. Our data demonstrate in vivo that the absence of Mstn impairs both mechanical performance and energy cost of contraction in hypertrophic muscle. These findings must be kept in mind when considering Mstn as a potential therapeutic target for increasing muscle mass in patients suffering from muscle-wasting disorders.

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MYOSTATIN (Mstn) is part of the transforming growth factor-β signaling molecule family and acts as an endogenous negative regulator of skeletal muscle growth in mammalians (23). The absence or postnatal blockade of Mstn results in a large and widespread increase in skeletal muscle mass (20, 23, 24). On that basis, Mstn has been proposed as a potential therapeutic target for increasing muscle mass in patients suffering from muscle-wasting disorders (24). However, the potential benefit of Mstn deficiency on muscle function and energetics remains a matter of debate. It is undisputable that the increased muscle size induced by the lack of Mstn is linked to an enhancement of absolute force generation that has been observed in vivo or in vitro in various murine muscles such as soleus (26), extensor digitorum longus (EDL) (26), and triceps surae (6). Also, chronic administration of an inhibitory antibody of Mstn in adult mice increases skeletal mass and grip strength (35). However, the corresponding improvement in specific force (i.e., absolute force scaled to muscle size) remains controversial. As an illustration, specific force in mouse models lacking Mstn has been reported to increase in triceps surae electrostimulated in vivo (6), to remain unaffected in isolated soleus muscle (26), or even to decrease in EDL (4, 26).

At the energy metabolism level, some in vitro studies in cultured mammalian myocytes and skeletal muscle extracts have reported that postnatal Mstn blockade alters basal activity of the main energy production pathways with an increase of creatine kinase (CK) activity (32) and acceleration of glucose uptake and utilization (24). Also, in vivo hyperinsulinemic euglycemic clamp experiments have evidenced increased glucose utilization in Mstn−/− mice (16). However, the resulting effects of Mstn deficiency at the whole body energy metabolism level warrant further investigation. Indirect calorimetry experiments have demonstrated a reduced basal energy expenditure in Mstn-deficient mice compared with control littermates, thereby suggesting an improved metabolic efficiency (24). On the contrary, similar experiments have reported an increased basal energy expenditure in Mstn−/− mice that could, by the way, account for the reduced body fat content reported in these mice (8) given that fatty acids are a major fuel in resting and exercising muscle (18). Although controversial, these data suggest that muscle energetics is disturbed in skeletal muscle lacking Mstn.

In the present study, we aimed at determining in vivo the effects of Mstn deficiency on skeletal muscle function and energetics. Mechanical performance, muscular volume, and energy production from the net breakdown of phosphocreatine (PCr) via CK reaction, oxidative phosphorylation, and anaerobic glycolysis were assessed strictly noninvasively in electrostimulated gastrocnemius muscle of Mstn−/− and wild-type (Mstn+/+) mice using an original experimental setup (14) implementing 1H-magnetic resonance (MR) imaging and 31P-MR spectroscopy.

MATERIALS AND METHODS

Ethics statements. Animal procedures were carried out in strict accordance with the guidelines of the European Communities Council Directive 86/609/EEC for Care and Use of Laboratory Animals and the French Law on the Protection of Animals (Decree No. 87-848). All experiments were performed (license no. 13.164 2008/11/25) in accordance with the guidelines of the European Communities Council Directive 86/609/EEC for Care and Use of Laboratory Animals and the French Law on the Protection of Animals (Decree No. 87-848). All experiments were performed (license no. 13.164 2008/11/25) in
the animal care facilities of the Direction Départementale des Services Vétérinaires des Bouches du Rhône, which also approved the experiments (agreement no. C-13-055). Every attempt was made to minimize the number and the suffering of animals used in this experiment.

Animal care and feeding. Thirteen 4-mo-old female Mstn+/− (n = 7) and Mstn−/− mice (n = 6) generated from a colony maintained on a C57Bl/6 background (Centre d’Expérimentation Fonctionelle, Faculté de Médecine Pitié Salpêtrière, Paris, France) were used for these experiments. Animals were socially housed four to six per cage in an environmentally controlled facility (12:12-h light-dark cycle, 22°C) with free access to commercial standard food and water until the time of the experiment. After the experiments, animals were euthanized by cervical dislocation following isoflurane anesthesia, and gastrocnemius muscles were quickly removed, freeze-clamped with liquid nitrogen-chilled metal tongs, and stored at −80°C for measuring ATP content.

Animal preparation. Mice were anesthetized initially in an induction chamber (Equipement Vétérinaire, Minerve, France) using 4% isoflurane in 33% O2 and 66% N2O. After the whole left hindlimb was shaved, electrode cream for electromyography was applied at the knee and heel regions to optimize electrical stimulation. Anesthetized animal was placed supine into a home-built cradle designed especially for the strictly noninvasive MR investigation of gastrocnemius muscle function (14). The setup integrates an ergometer consisting of a foot pedal coupled to a force transducer and two rod-shaped transcutaneous surface electrodes (one located above the knee and the other under the heel) connected to an electrical stimulator (Type 215/T; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). The foot was positioned on the ergometer pedal, and the lower hindlimb was centered inside a 20-mm-diameter 1H Helmholtz imaging coil while the belly of the gastrocnemius muscle was located above an elliptic (8 × 12 mm) 31P-MRS surface coil. The pedal position was adjusted to modify the angle between the foot and the lower hindlimb so that the gastrocnemius muscle could be passively stretched at rest to produce a maximum isometric twitch tension in response to supramaximal square wave pulses (1.5 ms duration; 2–4 mA). Corneas were protected from drying by applying ophthalmic cream, and the animal’s head was placed in a home-built facemask supplied continuously with 1.75% isoflurane in 33% O2 and 66% N2O (0.4 l/min) throughout the experiment for anesthesia maintenance. Animal body temperature was controlled and maintained at physiological temperature during anesthesia using a feedback loop, including an electrical heating blanket (Prang & Partner, Pfungen, Switzerland), a temperature control unit (ref. no. 507137; Harvard Apparatus), and a home-built rectal thermometer constructed with a NTC thermistor SMC series (ref no. NCP18XSW220J038-2.2K; Murata, Kyoto, Japan).

Muscle electrostimulation protocol and force measurement. The transcutaneous stimulation protocol consisted of 6 min of repeated maximal isometric contractions electrically induced with square wave pulses (2–4 mA; 1.5 ms duration) at a frequency of 1.7 Hz. Analog electrical signal coming out from the force transducer was amplified with a home-built amplifier (gain = 70 dB, 0–5 kHz bandwidth, Operational amplifier AD620; Analog Devices, Norwood, MA) and converted to a digital signal (PCI-6220; National Instrument, Austin, TX) that was monitored and recorded on a personal computer using the WinATs software version 6.5 (Sysma, Aix-en-Provence, France). Isometric force production was calculated by integrating the absolute isometric tension (in N) with respect to time and was expressed as tension-time integral (in N s).

MR data acquisition. Experiments were performed in the 4.7 T horizontal magnet of a 47/30 Biospec Avance MR system (Bruker, Karlsruhe, Germany) equipped with a Bruker 120-mm BGA12SL (200 mT/m) gradient insert. Ten consecutive noncontiguous axial scout slices (1-mm thickness, spaced 0.5 mm) covering the region from the knee to the ankle were selected across the lower hindlimb. RARE images of these slices (8 echoes; effective echo time = 67.9 ms, actual echo time = 16.7 ms, repetition time = 2,000 ms, 1 accumulation, 20 × 15 mm field of view, 256 × 256 matrix size) were recorded at rest. Spectra (8-kHz sweep width; 2,048 data points) from the gastrocnemius muscle region were acquired continuously throughout the standardized experimental protocol consisting of 6 min of rest, 6 min of stimulation, and 15 min of poststimulation recovery. MR data acquisition was gated to muscle stimulation to reduce potential motion artifacts due to contraction. A fully relaxed spectrum (12 scans, 20 s of repetition time) was acquired at rest, followed by a total of 768 saturated free induction decays (FID; 1.875 s of repetition time). The first 64 FIDs were acquired at rest and summed together. The next 192 FIDs were acquired during the stimulation period and were summed by packets of 32, allowing a temporal resolution of ~60 s. The remaining 512 FIDs were obtained during the poststimulation recovery period and summed as seven packets of 32 FIDs, followed by three packets of 64 FIDs and one packet of 96 FIDs.

MR data processing. MR data were processed using a custom-written image analysis program developed on the IDL software (Interactive Data Language; Research Systems, Boulder, CO). For each slice, regions of interest were outlined manually so that the corresponding cross-sectional areas (CSA) were measured. The gastrocnemius muscle volume was calculated as the sum of the four volumes included between the five consecutive slices. Relative concentrations of PCr, inorganic phosphate (Pi), and β-ATP were obtained by a time-domain-fitting routine using the AMARES-MRUI Fortran code (33) and appropriate prior knowledge of the ATP multiplets. Signal areas were corrected for magnetic saturation effects using fully relaxed spectra. Absolute concentrations of phosphorylated compounds were expressed relative to resting β-ATP concentrations of 5.18 ± 0.15 and 5.34 ± 0.15 mM for Mstn+/− and Mstn−/− animals, respectively. These concentrations were measured by high-performance liquid chromatography from extracts of freeze-clamped gastrocnemius muscles according to the previously reported protocol (12). Intracellular pH (pHi) was calculated from the chemical shift of the P, signal relative to PCr (5). Time points for the time course of pHi, and phosphorylated metabolite concentrations were assigned to the midpoints of the acquisition intervals.

Metabolic calculations. ATP production from CK reaction, mitochondrial oxidative phosphorylation, and glycolysis during the stimulation period were calculated according to quantitative interpretation of bioenergetics data (3, 13, 19). ATP cost of contraction referred to total ATP production scaled to force generation during the same time period. ATP production from the net breakdown of PCr via the CK reaction (D) was calculated directly using the PCr time course of PCr throughout the stimulation period: $D = -\frac{d[PCr]}{dt}$.

Oxidative ATP production (Q) was calculated on the basis of the assumption that oxidative ATP synthesis is controlled by ADP throughout a hyperbolic relationship: $Q = Q_{max}(1 + [ADP]/K_{m(ADP)})$, in which $K_m$ (the ADP concentration at half-maximal oxidation rate) is 50 μM, as reported previously in murine skeletal muscle (30), and $Q_{max}$ is the maximal oxidative capacity. ADP concentration was calculated considering the equilibrium of the CK reaction [ADP] = 10.2 ± 0.3 μM (H11002/H9262), with the equilibrium constant of the CK reaction ($K_{c}$) assumed to be 1.67 10$^6$ M$^{-1}$ (28). $Q_{max}$ was calculated using the rate of PCr resynthesis at the start of the poststimulation recovery period (VPCr$_{rec}$) and the concentration of free cytosolic ADP measured at the end of the stimulation period: $Q_{max} = VPCr_{rec} (1 + [ADP]_{obs})$. VPCr$_{rec}$ was the product of k (the pseudo-first-order rate constant of PCr recovery) and [PCr]$_{rest}$ (the amount of PCr consumed at the end of the stimulation period). To determine k, the PCr time course during the poststimulation recovery period was fitted to a first-order exponential curve: [PCr]$_{rest} = [PCr]_{rest} - [PCr]_{obs} e^{-kt}$, where [PCr]$_{rest}$ is the concentration of PCr measured at rest.

Glycolytic ATP synthesis (L) was inferred by considering that, when coupled to ATP hydrolysis, glycolytic ATP production is related to
to proton synthesis (H_{CK}), with a stoichiometry of 1.5 mol ATP per proton (L = 1.5 H_{CK}) (17). Indeed, the degradation of each mol of glycosyl unit generates 3 mol of ATP, whereas the hydrolysis of 3 mol of ATP is coupled to the production of 2 mol of protons (17). This proton production can be calculated from the observed changes in pHi of ATP is coupled to the production of 2 mol of protons (17). This apparent buffering capacity (β_{app}) takes into account the buffering capacity of P_{i} (β_{P}) and the buffering capacity of muscle tissue (β_{tissue}): β_{total} = β_{P} + β_{tissue}, where β_{P} = 2.3[|P_{i}|/(1 + 10^{6.75 - p[H]}]) (36). It has been demonstrated that β_{tissue} varies linearly between pH 7 (16 Slykes) and pH 6 (37 Slykes) in murine gastrocnemius muscle (1). Accordingly, β_{tissue} was calculated as follows: β_{tissue} = -21 pHi + 163. During muscle stimulation, H_{efflux} was calculated using the proportionality constant λ (in mM·min^{-1}·pH unit^{-1}), referring to the ratio between the rate of proton efflux and pH: H_{efflux} = λΔpHi. This constant was determined at the start of the poststimulation recovery period as λ = -V_{max}/ΔpHi. At that time, although protons were generated throughout the aerobic PCr resynthesis, pHi recovered back to basal because of net proton efflux from the cell; H_{efflux} can then be calculated, taking into account proton loads associated with CK reaction and mitochondrial ATP synthesis on the one hand and the rate of pH changes on the other hand. H_{efflux} = H_{CK} + H_{OX} + β_{total} dpHi/dt. The rate of aerobic proton production coupled to oxidative ATP synthesis was quantified as described previously (36): H_{OX} = mVCrrec, with m = 0.16/(1 + 10^{6.75 - p[H]}).

**Statistics.** Values are expressed as means ± SE. Data were analyzed with paired or unpaired two-tailed Student’s t-test (JMP software; SAS Institute, Cary, NC). A P value of <0.05 was considered significant.

**RESULTS**

**Physiological changes.** Both body weight (20.4 ± 0.4 g in Mstn^{+/+} vs. 28.2 ± 0.7 g in Mstn^{-/-}) and gastrocnemius muscle volume (84.8 ± 1.9 mm³ in Mstn^{+/+} vs. 184.8 ± 3.4 mm³ in Mstn^{-/-}) were larger (+38 and +118%, respectively) in Mstn^{-/-} animals compared with Mstn^{+/+} ones.

**Muscle force measurement.** For each group, both absolute and specific forces transiently increased in the early stage of the stimulation period to reach a maximal value, after which they progressively decreased until the end of the stimulation period as a sign of fatigue development (Fig. 1, A and B); at this stage, the extent of force reduction was larger (P = 0.023) in Mstn^{-/-} animals compared with Mstn^{+/+} (Fig. 2A). Total absolute force developed during the whole 6-min stimulation period was larger (+35%, P = 0.005) in the Mstn^{-/-} group (Fig. 2B), but total specific force was lower (−36%, P < 0.001; Fig. 2C).

**Phosphorylated compounds and pH.** At rest, Mstn^{-/-} mice did not display significant alteration of [PCr] (14.6 ± 0.8 mM in Mstn^{+/+} vs. 16.2 ± 1.0 mM in Mstn^{-/-}; Fig. 3A), [Pi] (2.1 ± 0.4 mM in Mstn^{+/+} vs. 1.8 ± 0.2 mM in Mstn^{-/-}; Fig. 3B), pHi (7.10 ± 0.04 in Mstn^{+/+} vs. 7.16 ± 0.06 in Mstn^{-/-}; Fig. 3C), [ATP] (5.2 ± 0.2 mM in Mstn^{+/+} vs. 5.3 ± 0.2 mM in Mstn^{-/-}; Fig. 3D), or [PCr]/[ATP] ratio (Table 1). At the
Onset of the stimulation protocol, PCr was rapidly degraded (Fig. 3A) at a similar rate between both groups (Table 1). In the middle of the stimulation period, [PCr] reached a steady state that was maintained until the end of the stimulation period; at that time, the extent of PCr consumption was larger in Mstn/+/+ animals (Table 1). The time course of Pi during the stimulation period exhibited an initial phase of rapid and massive accumulation, followed by a phase of steady state (Fig. 3B); at the end of the stimulation period, [Pi] amounted to 12.0 ± 0.7 and 15.0 ± 1.1 mM in Mstn+/+/+ and Mstn−/− mice, respectively. Intracellular pH fell rapidly in the early stage of the stimulation period (Fig. 3C). After 3 min of stimulation, it reached a steady state and remained fairly constant until the end of the stimulation period in Mstn+/+/+ animals. On the contrary, a continuous pHi drop was recorded for Mstn−/− animals so that the end-of-stimulation ΔpHi was 63% larger compared with Mstn+/+ (Table 3).

Fig. 3. Changes in gastrocnemius phosphocreatine concentration ([PCr]; A), [Pi] (B), intracellular pH (pHi; C), and [ATP] (D) during 6-min stimulation and 15-min poststimulation recovery periods. Time points were assigned to the midpoints of the acquisition intervals. The first time point (t = 0) indicates the resting value. Data are means ± SE.
Table 1. Energy metabolism in mouse gastrocnemius muscle

<table>
<thead>
<tr>
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<th>Mstn+/+</th>
<th>Mstn−/−</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Basal [PCr]/[ATP]</td>
<td>2.81 ± 0.16</td>
<td>3.02 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Onset of the stimulation period</td>
<td>9.5 ± 1.4</td>
<td>12.0 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>End of the stimulation period</td>
<td>63.0 ± 2.7</td>
<td>75.1 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>6.72 ± 0.04</td>
<td>6.53 ± 0.04</td>
<td>0.005</td>
</tr>
<tr>
<td>ΔpH, (pH units)</td>
<td>0.38 ± 0.07</td>
<td>0.62 ± 0.09</td>
<td>0.035</td>
</tr>
<tr>
<td>Poststimulation recovery period</td>
<td>4.50 ± 0.81</td>
<td>4.99 ± 0.82</td>
<td>NS</td>
</tr>
<tr>
<td>VPCR, mm/min</td>
<td>11.3 ± 1.1</td>
<td>13.8 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>VPCRrec, mm/min</td>
<td>4.17 ± 0.54</td>
<td>6.58 ± 0.95</td>
<td>0.030</td>
</tr>
<tr>
<td>V, mM·min⁻¹·pH unit⁻¹</td>
<td>13.2 ± 3.2</td>
<td>11.6 ± 2.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; NS, not statistically significant; VPCRstim, initial rate of phosphocreatine (PCr) breakdown at the start of the 6-min stimulation period; PCrcons, PCr consumption at the end of 6-min stimulation period; pH, intracellular pH; VPCRrec, initial rate of PCr resynthesis at the end of the poststimulation period; Qmax, maximal oxidative capacity; VHrec, initial rate of proton efflux at the start of the poststimulation period; λ, proportionality constant relating proton efflux to pH.

ATP production at the onset and at the end of the 6-min stimulation period. Data are means ± SE, **P < 0.01 vs. Mstn+/+. Table 2. ATP production (mM/min) at the onset (1st min) and at the end (6th min) of the 6-min stimulation protocol

<table>
<thead>
<tr>
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<th>Mstn+/+</th>
<th>Mstn−/−</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of stimulation ATP from creatine kinase reaction</td>
<td>7.1 ± 1.0</td>
<td>8.7 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Glycolytic ATP</td>
<td>7.7 ± 2.1</td>
<td>10.1 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Oxidative ATP</td>
<td>3.2 ± 0.4</td>
<td>4.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total ATP</td>
<td>18.0 ± 2.1</td>
<td>23.4 ± 1.2</td>
<td>0.039</td>
</tr>
<tr>
<td>End of stimulation ATP from creatine kinase reaction</td>
<td>0.05 ± 0.09</td>
<td>−0.05 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Glycolytic ATP</td>
<td>4.5 ± 1.2</td>
<td>10.6 ± 1.9</td>
<td>0.011</td>
</tr>
<tr>
<td>Oxidative ATP</td>
<td>4.5 ± 0.8</td>
<td>5.0 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total ATP</td>
<td>9.1 ± 1.6</td>
<td>15.5 ± 2.2</td>
<td>0.022</td>
</tr>
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Data are means ± SE.

DISCUSSION

This in vivo study provides original information about the metabolic impact of the lack of Mstn expression in skeletal muscle and, as a matter of consequence, new insights into the functional role exerted by Mstn. We reported mainly that both force generation and ATP cost of contraction were impaired in hypertrophic muscle from Mstn-deficient mice. Also, glycolytic contribution to energy production in contracting muscle was larger in these animals, whereas oxidative capacity was unaffected.

The larger body weight and gastrocnemius muscle volume (+38 and +118%, respectively) in Mstn−/− animals are consistent with previous experiments showing that Mstn−/− mice have two- to threefold greater muscle mass than their Mstn+/+ littermates (35). The fact that muscle volume increase was almost three times larger than the gain in body weight could be explained partially by an increased relative contribution of lean mass in Mstn−/− mice. Muscle hypertrophy resulting from the lack of postnatal blockade of Mstn expression is thus associated with a reduced body fat accumulation (20, 23, 24), most likely because hypertrophied muscle favors fat utilization for growth and maintenance (37).

Our data show evidence that muscle hypertrophy induced by Mstn deficiency reduces force-generating capacity. Actually, the greater gastrocnemius muscle volume in Mstn−/− mice was not accompanied by a proportionate increase in absolute force (+35%), and specific force was further lower (−36%). Our findings obtained in vivo corroborate previous in vitro experiments showing a decreased specific force in EDL muscle of Mstn−/− mice (4, 25) and are in line with the common view that the net force per CSA decreases in a muscle undergoing hypertrophy or hyperplasia (26); however, they are in opposi-
increased glycolytic fiber content reaches 20% thereby leading to an overall faster and more glycolytic muscle oxidative and oxidative/glycolytic ones in hindlimb muscles, relative fraction of glycolytic fibers at the expense of both clarification. Mstn deficiency is known for increasing the previous studies in Mstn-deficient animals warrants further technique is considered a gold standard in this field (11, 31).

Muscle performance impairment reported herein and in previous studies in Mstn-deficient animals warrants further clarification. Mstn deficiency is known for increasing the relative fraction of glycolytic fibers at the expense of both oxidative and oxidative/glycolytic ones in hindlimb muscles, thereby leading to an overall faster and more glycolytic muscle phenotype. This increased glycolytic fiber content reaches 20% in gastrocnemius muscle (6), 15–25% in soleus muscle (10, 15), 25–45% in EDL (4, 15), and 23 and 32% in tibialis anterior and plantaris, respectively (22). Given the lesser fatigue resistance of glycolytic fibers compared with oxidative ones, these typological changes may explain the larger fatigability we and others measured in Mstn\(^{-/-}\) mice. These animals indeed display a lower endurance capacity for running and swimming (22, 29).

Noteworthy, specific force reduction in Mstn-deficient mice is paradoxical because glycolytic fibers (in larger proportion in these animals) are expected to produce more force compared with oxidative fibers. An attractive explanation for this phenomenon would lie in the shift of the fiber-type recruitment pattern since fatigue development in mixed muscle such as gastrocnemius is associated with a decreased recruitment of glycolytic fibers at the expense of oxidative fibers, which are more fatigue resistant (7, 13). However, such an issue can be ruled out herein because specific force produced by Mstn\(^{-/-}\) animals was lower as soon as the onset of the electrical stimulation period, i.e., before fatigue development. At this stage, it is unlikely that oxidative fibers would be preferentially recruited inasmuch as the basic characteristic of electrical stimulation is to elicit the recruitment of all types of motor units (2, 21). Perhaps muscular architecture and/or calcium flux play a role in this muscle performance impairment. Massive hypertrophy is considered to alter the angle of pull on the muscle fibers during contraction, hence reducing the ability to generate a higher force (4, 26, 27). Although our experimental setup enabled us to work at muscle optimal length, alterations in fiber penetration, if any, cannot be taken into account. Besides, histological analyses of fast muscle fibers from adult Mstn\(^{-/-}\) mice have shown evidence of an accumulation of tubular aggregates at the calcium release channel of the sarcoplasmic reticulum that could alter calcium handling during excitation-contraction cycles (4).

Mstn deficiency was also associated with marked alterations in muscle energetics. Whereas phosphorylated compound content and \(pH_1\) were similar at rest between Mstn\(^{-/-}\) and Mstn\(^{+/+}\), animals as reported previously (6), both PCr consumption and acidosis were larger in contracting Mstn\(^{-/-}\) muscle, thereby illustrating a higher intramuscular energy demand. We actually found that ATP production was significantly higher in Mstn\(^{-/-}\) animals, notably at the end of the stimulation period when glycolytic ATP production was 135% larger compared with Mstn\(^{+/+}\) animals. According to the quantitative interpretation of bioenergetics data (3, 13, 19), this glycolytic flux enhancement was due mainly to the larger changes in \(pH_1\) during the stimulation period and is in agreement with the acceleration of glucose metabolism through increased glucose uptake and utilization in Mstn\(^{-/-}\) mouse muscle (24).

In Mstn-deficient mice, the combination between reduced force-generating capacity and increased ATP production results in a large increase in ATP cost of contraction. This increase reaches up to \(+206\%\) compared with Mstn\(^{+/+}\) and corresponds to a 66% decrease in contractile efficiency in Mstn\(^{-/-}\) animals. In other words, hypertrophic muscles of these animals need more energy to produce a given amount of force. This increased contractile cost is in line with the fact that Mstn\(^{-/-}\) muscle contains more fast fibers that are known to produce more force at the cost of consuming more ATP because of faster cross-bridge cycling kinetics as well as increased size (9). Overall, this ATP wasting is consistent with

**Fig. 5.** Raw ATP production (A), absolute force (B), and ATP cost of contraction (C) at the onset and at the end of the 6-min stimulation period. Data are means \(\pm SE\). *\(P < 0.05\) vs. Mstn\(^{+/+}\); **\(P < 0.01\) vs. Mstn\(^{+/+}\); ***\(P < 0.001\) vs. Mstn\(^{+/+}\).
previous indirect calorimetry experiments demonstrating an increased basal energy expenditure in Mstn-deficient mice (8).

Such an increased ATP cost of contraction in Mstn-deficient muscle has never been described so far, and from a careful analysis of the literature, we hypothesized that at least two mechanisms are involved. First, the higher proportion of glycolytic fibers in Mstn-/- muscle could directly account for the increased ATP cost of contraction given that these fibers contract less economically than oxidative ones (9). Second, Mstn deficiency is related to a decreased expression of several collagen genes, resulting in a reduction of collagen content per milligram of muscle tissue (26, 34). This reduction would increase the stiffness of aponevrosis and tendon, thus limiting the ability to generate a higher force output, as suggested previously (4, 25). In that context, an increased amount of ATP would be wasted to overcome mechanical resistance in contracting Mstn-deficient muscle.

Another interesting finding is that Mstn deficiency modifies the balance between oxidative and anaerobic energy production in exercising muscle. At the onset of the stimulation period, we found that oxidative and anaerobic processes contributed to around 20 and 80% of ATP production, respectively, in both groups, which is consistent with the view that the balance between oxidative and anaerobic energy production and a recruitment of less economic and less fatigue-resistant glycolytic fibers. These data should be kept in mind when considering Mstn as a potential therapeutic target for increasing muscle mass in patients suffering from muscle-wasting disorders.

DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, to declare.

AUTHOR CONTRIBUTIONS

B.G. and D.B. contributed to the conception and design of the research; B.G. and C.V. performed the experiments; B.G. analyzed the data; B.G. and D.B. interpreted the results of the experiments; B.G. prepared the figures; B.G. drafted the manuscript; B.G., H.A., M.B. and D.B. edited and revised the manuscript; B.G., C.V., H.A., M.B. and D.B. approved the final version of the manuscript.

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