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The effect of constitutive inactivation of the myostatin gene on the gain in muscle strength during postnatal growth in two murine models

Amalia Stantzou PhD[1], Vanessa Ueberschlag-Pitiot PhD[2], Remi Thomasson PhD[3], Denis Furling PhD[4], Anne Bonnieu PhD[5], Helge Amthor PhD [1], Arnaud Ferry PhD [4,6]

1- Université de Versailles Saint-Quentin, Unité de formation et de recherche des sciences de la santé des sciences, Inserm U1179, LIA BAHN CSM Montigny-le-Bretonneux, France.

2- Université de Strasbourg, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104/INSERM U964, Illkirch Cedex, France

3-Université Paris Descartes, Institut de Recherche bio-Médicale et d'Epidémiologie du Sport, Sorbonne Paris Cité, Paris, France.

4-Sorbonne Universités UPMC Univ Paris 06, Inserm, CNRS, Centre de Recherche en Myologie, Paris, France

5- INRA, Université Montpellier, UMR 866 Dynamique Musculaire et Métabolisme, Montpellier, France

6-Université Paris Descartes, Sorbonne Paris Cité, Paris, France.

Maximal force without myostatin

Correspondance :

A. Ferry

G.H. Pitié-Salpétrière, 47, bld de l'Hôpital, Bâtiment Babinski, Centre de recherche en myologie , 75651 Paris cedex 13, France.

arnaud.ferry@upmc.fr

Abstract

Introduction. The effect of constitutive inactivation of the gene encoding myostatin on the gain in muscle performance during postnatal growth hsa not been well characterized. Methods. We analyzed 2 murine myostatin knockout (KO) models: i) the Lee model (KO^{Lee}) and ii) the Grobet model (KO^{Grobet}), and measured the contraction of tibialis anterior muscle *in situ*. Results. Absolute maximal isometric force was increased in 6-month old KO^{Lee} and KO^{Grobet} mice, as compared to wild-type mice. Similarly, absolute maximal power was increased in 6-month old KO^{Lee} mice. In contrast, specific maximal force (relative maximal force **per unit of muscle mass** was decreased in all 6-month old male and female KO mice, except in 6-month old female KO^{Grobet} mice, whereas specific maximal power was reduced only in male KO^{Lee} mice. Discussion. Genetic inactivation of myostatin increases maximal force and power, but in return it reduces muscle quality, particularly in male mice.

Keywords

Skeletal muscle; postnatal growth; knockout; myostatin; force; power; castration; orchidectomy

Introduction

Myostatin, a member of the transforming growth factor beta family of signaling molecules, is a negative regulator of skeletal muscle growth. The gene encoding myostatin (*Mstn*) is expressed in both developing and adult muscle (1; 2). In 2 mouse models and 1 rat model of constitutive *Mstn* knockout (KO), it has been reported that skeletal muscle undergoes marked hypertrophy (2–4). The supplementary Table S1 (available online) highights the increase in muscle weight, an index of muscle hypertrophy, that results mainly from hyperplasia in KO adult rodents (2; 4–6). Many studies have examined muscle strength, i.e. absolute maximal force (tetanic isometric force), in KO adult rodents. The findings are extremely variable, since absolute maximal force has been reported to be increased (4; 6; 7), unchanged (8–10), or even reduced (11) as compared to wild-type rodents. As shown in supplementary Table S1, the discrepancy in absolute maximal force appears to be more related to differences in the reduction of specific maximal force, i.e. absolute maximal force relative to muscle size, than to an increase in muscle weight (hypertrophy). Alternatively, the discrepancies between the studies may be due to methodological differences in force measurements (*in situ* versus *in vitro,* or nerve versus muscle stimulation), or to differences in ages, sexes, and genetic models.

The general aim of this study was to systematically determine the effect of constitutive genetic inactivation of myostatin on muscle performance gain during postnatal growth by analyzing different stages of postnatal development. We first analyzed absolute maximal force and absolute maximal power in 4 week-, 6 week-, 3 month-, and 6 month-old KO mice, using the mouse model generated by the Lee group to constitutively inactivate *Mstn* (2) (referred to hereafter as KO^{Lee} mice). It is not yet known at what age the performance gain induced by *Mstn* inactivation occurs. We also evaluated whether the effect of genetic

inactivation of myostatin varies between murine models. To this end, mice from the Grobet model of constitutive *Mstn* inactivation (3) (referred to hereafter as KO^{Grobet} mice) were studied and compared to KO^{Lee} mice. Moreover, we analyzed the effect of castration in order to determine the effect of *Mstn* inactivation in the absence of sex hormones, which are additional potential regulators of muscle performance. In this study, mice of both sexes were studied since there have been reports of differences between sexes regarding the effect of *Mstn* inactivation on muscle performance (7; 9).

Materials and Methods

Animals

All procedures were performed in accordance with national and European laws **and were approved by your institutional animal care and use committee**. Mice were group-housed in plastic cages and maintained on a standard chow-diet with a 12 h light and dark cycle, with 21°C room temperature.Two mouse models with constitutive *Mstn* inactivation were analyzed. We studied 61 female and 48 male KO^{Lee} mice, which were compared to 45 female and 61 male wild-type (WT) mice. KO^{Lee} mice founder breeding pairs were a gift from Se-Jin Lee (2). The KO^{Lee} mice carry a deletion of the entire mature C-terminal region (which comprises the third exon of the *Mstn* gene), which is replaced by a neo cassette. We used 10 female and 8 male KO^{Grobet} mice which were compared to 8 female and 7 male WT mice. We also studied some heterozygotous mice (HTZ^{Lee} mice). KO^{Grobet} mice founder breeding pairs were provided by Luc Grobet (3). The KO^{Grobet} mice carry the floxed *Mstn* allele (where the third exon of the *Mstn* gene is flanked with a pair of loxP sites) that was deleted at the zygote stage by a Cre plasmid. Both mouse models had a C57BL/6

background. Some female and male KO^{Lee} mice (n=24 and n=19 respectively), as well as female and male WT mice (n=14 and n=15 respectively) were castrated (surgical removal of gonads) before age 4 weeks, approximately corresponding to the beginning of puberty (12). At age 6 months, body weights were 35.5 ± 0.2g (castrated 32.9 ± 0.3g) in male KO^{Lee} mice, 30.0±0.5g (castrated 30.9 ± 1.1g) in male wildt-type (WT) mice, 29.8 ± 0.4g (castrated 30.0 ± 0.6g) in female KO^{Lee} mice, 25.4 ± 0.4 g (castrated 30.4±1.1g) in WT female mice.

Muscle performance

Force and power were evaluated by measuring *in situ* tibialis anterior (TA) muscle contraction in response to nerve stimulation, as described previously (13; 14). Mice were anesthetized using pentobarbital (60 mg/kg injected intraperitoneally). Body temperature was maintained at 37°C using radiant heat. The knee and foot were fixed with pins and clamps, and the distal tendon of the muscle was attached to a lever arm of a servomotor system (305B, Dual-Mode Lever, Aurora Scientific) using a silk ligature. The sciatic nerve was crushed proximally and stimulated distally by a bipolar silver electrode using 0.1 ms duration supramaximal square wave pulses. We measured the absolute maximal force (P0) that was generated during isometric contractions in response to electrical stimulation (frequency, 75–150 Hz, 500 ms stimulus train). Absolute maximal force was determined at L0 (length at which maximal tension was obtained during the tetanus). Absolute maximal force was normalized to the muscle mass as an estimate of specific maximal force (sP0), i.e. relative force-generating capacity **per unit of muscle mass.**

Force-velocity data were then obtained by eliciting contractions in response to sciatic nerve stimulation (500 ms, 125 Hz) at 6 different afterloads over the range of approximately 10-

50% absolute maximal force. The sciatic nerve was stimulated for 700 ms (125 Hz). A maximal isometric contraction of the muscle was initiated during the first 200 ms. Then, the muscle shortened during the last 300 ms against the load. Each contraction was separated by a 1 min resting period. The (peak) shortening velocity was measured during the first 20 ms of the shortening period. The absolute power was calculated from the force-velocity data, and the absolute maximal power was reported (Pmax). Specific maximal power (sPmax) was calculated by dividing maximal power by muscle weight. After contractile measurements, the animals were sacrificed by cervical dislocation, and muscles were **removed and** weighed.

Statistical analysis

Groups were generally compared using 2-way analysis of variance (genotype x age, sex x age, sex x genotype, castration x age) of variance. If necessary, Bonferroni post-tests were also performed. For groups that did not pass tests of normality and equal variance, non-parametric tests were used (Kruskal Wallis and Wilcoxon). Values are reported as mean ± standard error of the mean (SEM). Significance was set at $P < 0.05$.

Results

Effect of Mstn inactivation: Lee model

There were significant ($P < 0.05$) effects of both age and genotype on absolute maximal force and interaction between factors in male KO^{Lee} mice. The analyses revealed that absolute maximal force increased with age ($P < 0.05$) and peaked at ages 3 and 6 months in

male KO^{Lee} mice and at age 6 months in male WT mice (Figure 1A). Moreover, absolute maximal force was increased in 3-month old male KO^{Lee} mice (+45%) and 6-month old male KO^{Lee} mice (+41%), as compared to age-matched male WT mice (Figure 1A). However, absolute maximal force was decreased in 6-week old male KO^{Lee} mice compared to WT (Figure 1A). In contrast to male KO^{Lee} mice, absolute maximal force was only increased in 6-month old female KO^{Lee} mice (+55%), but not at age 3 months, as compared to age-matched female WT mice (Figure 1B). Gender dimorphism was also studied by a 2-way analysis of variance (gender x age) in KO^{Lee} and WT mice. The analyses revealed that, in contrast to WT mice, only the absolute maximal force of 3-month old male KO^{Lee} mice (Figure 1A) was increased compared to age-matched female KO^{Lee} mice (Figure 1B).

Specific maximal force (absolute maximal force/muscle weight) was decreased in 4-week, 6-week, 3- (-23%), and 6-month old (-34%) male KO^{Lee} mice, as compared to age-matched male WT mice (Figure 1C). Similarly, specific maximal force was reduced in female KO^{Lee} mice at ages 6 weeks, 3 months (-30%), and 6 months (-20%), as compared to age-matched female WT mice (Figure 1D). Moreover, in contrast to WT mice, the specific maximal force of male KO^{Lee} mice (Figure 1C) was reduced as compared to age-matched female KO^{Lee} mice (Figure 1D).

Another important aspect of muscle performance is the absolute maximal power, a more dynamic measure that also accounts for shortening velocity. Absolute maximal power increased with age (P <0.05) and peaked at ages 3 and 6 months in male KO^{Lee} and WT mice. Three-month (+ 61%) and 6-month (+72%) old male KO^{Lee} mice had a greater absolute maximal power than age-matched male WT mice (Figure 2A). In contrast to male KO^{Lee} mice, absolute maximal power was only increased in 6-month old female KO^{Lee} mice

(+97%), but not in 3-month-old female KO^{Lee} mice, as compared to age-matched female WT mice (Figure 2B). Moreover, in contrast to WT mice, only the absolute maximal power of 3-month old male KO^{Lee} mice (Figure 2A) was increased as compared to age-matched female KO^{Lee} mice (Figure 2B).

Absolute maximal power was related to specific maximal power and muscle weight (see below). Specific maximal power was reduced in 4-week, 3-, and 6-month old (-22%) male KO^{Lee} mice, as compared to age-matched male WT mice (Figure 2C). In contrast to male KO^{Lee} mice, specific maximal power was not reduced in female KO^{Lee} mice, as compared to age-matched female WT mice (Figure 2D). Moreover, in contrast to WT mice, the specific maximal power of male KO^{Lee} mice was not increased (Figure 2C), as compared to age-matched female KO^{Lee} mice (Figure 2D).

We also analyzed muscle weight, because force and power are proportional to muscle size (muscle cross-section area and volume). Muscle weights increased with age (P <0.05) and peaked at ages 3 and 6 months in both male KO^{Lee} and WT mice. The muscle weight of 3- and 6-month old male KO^{Lee} mice was greater (+83% and +114%), as compared to age-matched male WT mice (Figure 3A). Similarly to male KO^{Lee} mice, muscle weight was increased in female KO^{Lee} mice at ages 3 and 6 months (+67% and +96%), as compared to age-matched female WT mice (Figure 3B). However, in contrast to male KO^{Lee} mice, muscle weight was also increased in female KO^{Lee} mice at age 4 weeks, as compared to age-matched female WT mice (Figure 3B). Moreover, similarly to WT mice, the muscle weight of 3- and 6-month old male KO^{Lee} mice was greater (Figure 3A), as compared to age-matched female KO^{Lee} mice (Figure 3B).

Effect of inactivation of a single Mstn allele: Lee model

We also studied the effect of inactivation of a single copy of the *Mstn* allele in heterozygous (referred to as HTZ^{Lee}) mice in the Lee model at age 3 months. Similarly to 3-month old male KO^{Lee} mice, absolute maximal force was increased in 3-month old male HTZ^{Lee} mice, as compared to age-matched male WT mice (Figure 4A). In contrast to 3-month old KO^{Lee} mice, specific maximal force was not reduced in 3-month old male and female HTZ^{Lee} mice, as compared to age- and sex-matched WT mice (Figure 4A,B). In contrast to 3-month old KO^{Lee} mice, muscle weight was less or not increased in 3-month old male and female HTZ^{Lee} mice, respectively, as compared to age- and sex-matched WT mice (Figure 4A,B). Together, these results suggest a dose-dependent effect of *Mstn* inactivation.

Effect of Mstn inactivation in castrated mice (Lee model)

Mice were castrated at age 4 weeks in both male and female KO^{Lee} mice, and they were studied at ages 3 and 6 months. In contrast to intact male KO^{Lee} mice (not castrated) (Figure 1A), absolute maximal force was increased (+63%) in 3-month old castrated male KO^{Lee} mice but not at age 6 months, as compared to age-matched castrated male WT mice (Figure 5A). Similarly to intact male KO^{Lee} mice (Figures 2A and 3A), absolute maximal power and muscle weight were increased in 3- and 6-month old castrated male KO^{Lee} mice (+100%, +91% respectively), as compared to age-matched castrated WT mice (Figures 5BC).

In contrast to intact female KO^{Lee} mice (Figures 1B and 2B), absolute maximal force and absolute maximal power were increased in both 3-and 6-month old castrated female KO^{Lee} mice, as compared to age-matched castrated female WT mice (Figure 5DE). Similarly to

intact female KO^{Lee} mice (Figures 3B), muscle weight was increased in 3- and 6-month old castrated female KO^{Lee} mice, as compared to age-matched castrated WT mice (Figure 5F).

Taken together, these results suggest that castration modulates some effects of *Mstn* inactivation.

Effect of Mstn inactivation: Grobet Model

Two-way analyses of variance (genotype x gender; genotype x age) were performed. The latter revealed that absolute maximal force was increased in 6-month old male and female KO^{Grobet} mice, as compared to sex-and age-matched WT mice (Figure 6A). In contrast to male KO^{Grobet} mice (+16%), absolute maximal force increased more markedly in 6-month old female KO^{Grobet} mice (+65%), as compared to age-matched female WT mice (Figure 6A). Absolute maximal force was also increased in 6 week-old female KO^{Grobet} mice (Figure 6A). Moreover, in contrast to WT mice, the absolute maximal force was not increased in 6-month old male KO^{Grobet} mice, as compared to age-matched female KO^{Grobet} mice (Figure 6A).

Specific maximal force was decreased in 6-month old male KO^{Grobet} mice (-35%), as compared to age-matched male WT mice (Figure 6B). In contrast to male KO^{Grobet} mice, specific maximal force was not reduced in 6-month old female KO^{Grobet} mice, as compared to age-matched female WT mice (Figure 6B) ($P = 0.055$). However, specific maximal force was reduced in female KO^{Grobet} mice (-33%) at age 6 weeks. There were no other signs of gender dimorphism.

The muscle weight of 6-month old male and female KO^{Grobet} mice was greater by +73% and +87%, respectively as compared to sex-and age-matched WT mice (Figure 6C). Moreover, muscle weight was also increased in 6-week old female KO^{Grobet} mice (Figure 6C). Furthermore, in both genotypes, the muscle weight of 6-month old male mice was greater as compared to age-matched female mice (Figure 6C).

We next compared the effect of *Mstn* inactivation between the Lee and Grobet models. Table 1 shows the effect of *Mstn* inactivation on muscle weight, absolute maximal force, and specific maximal force in KO^{Lee} and KO^{Grobet} mice of both sexes at age 6 months. Absolute maximal force increased in both sexes and models (P <0.05), but to a lower extent in male KO^{Grobet} mice. Specific maximal force decreased in both male and female KO^{Lee} mice and male KO^{Grobet} mice (P <0.05), but not in female KO^{Grobet} mice. In both models, muscle weight increased in male and female KO mice ($p < 0.05$). Together, these results indicate some differences between the 2 models.

Discussion

Mstn inactivation increases absolute maximal force/power in 6-month old mice

A main finding of our study is that inactivation of the *Mstn* gene increased absolute maximal force at age 6 months in both models and sexes, in line with some but not all previous studies (see supplementary table). However, the extent of the increase varied markedly (+16% to +65%), depending on the model and sex. Absolute maximal power was also increased in 6-month old KO mice, at least in KO^{Lee} mice. However, the general notion that *Mstn* inactivation increases maximal force/power may be incorrect in younger KO^{Lee} mice

since, for example, absolute maximal force was found unchanged in 3-month old female KO^{Lee} mice and was reduced in 6-week old male KO^{Lee} mice.

Interestingly, our study indicates that the increase in maximal force and power occurs late during postnatal growth in the Lee model (at ages 3 and 6 months). This is apparently not the case in the Grobet model, in which a notable absolute maximal force gain was observed in 6-week old female KO^{Grobet} mice. The explanation of this delayed effect in the Lee model can likely be explained by the fact that no substantial muscle hypertrophy (increased weight) occured until age 3 or 6 months in KO^{Lee} mice, in contrast to the Grobet model. This result suggests that the effect of *Mstn* inactivation on muscle size in the Lee model only occurs during the postnatal growth stage in which fiber growth is achieved without myonuclear accretion (beyond age 3 weeks) (15).

Mstn inactivation reduces specific maximal force

We also found that *Mstn* inactivation reduces specific maximal force (-20 to -35%), i.e. relative force generating capacity per unit of muscle mass (muscle quality), except in 6-month old female KO^{Grobet} mice (see Table 1). These results are in line with several previous studies (see supplementary Table). The fact that specific maximal force was not reduced in 6-month old female KO^{Grobet} as compared to age-matched female WT mice ($p = 0.055$) has to be confirmed since younger female KO^{Grobet} mice exhibited a reduction (-33%) in specific maximal force. Moreover, we detected a *Mstn* gene dose effect, as inactivation of a single *Mstn* gene allele is not sufficient to lead to a decrease in the specific maximal force (no reduction in HTZ^{Lee} mice). It is noteworthy that the reduced specific maximal force decreases the potential gain in absolute maximal force (see above) that can be expected on

the basis of muscle hypertrophy in both models (see Table 1).

An interesting finding was that the lower specific maximal force occurs very early during postnatal growth (4 or 6 weeks). Another important result of this study is that the reduced specific maximal force is not related to muscle hypertrophy, since notable hypertrophy occurs later, at least in the Lee model (in 3-month old male and female KO^{Lee} mice).

Previous studies have shown that *Mstn* inactivation reduces specific maximal force produced by individual permeabilized muscle fibers when activated by external calcium (16; 17). Moreover, a recent study reported a reduction in electrically evoked calcium release in mice with a mutation in *Mstn* probably due to reduction of calcium content in the sarcoplasmic reticulum (18). Therefore, one can postulate that the reduced specific maximal force in both models is likely caused by decreased myofibrillar function, and a dysfunction of sarcoplasmic calcium release. Since we found no reduced specific maximal force in 6-month old female KO^{Grobet}, we hypothesise that in these mice there is no dysfunction. The lower muscle quality resulting from *Mstn* inactivation is also evidenced by reduced specific maximal power, at least in male KO^{Lee} mice. Since it has been shown that exercise training can normalize specific maximal force in KO^{Lee} mice (11), it is possible that the reduced muscle quality results from a lower amount of habitual activity. However, a recent study failed to demonstrate any reduction in the level of home cage voluntary locomotor activity in KO^{Lee} mice (19). It is noteworthy that inactivation of myostatin has been shown to induce a fiber type shift (8). In future studies, it would be of interest to determine the effect of *Mstn* inactivation on the work loop power output, since the work loop technique relates well to the dynamic muscle performance *in vivo* (20).

Gender differences and effect of castration

We found several differences between sexes at ages 3 and 6 months in KO mice of both models, i.e. in mature mice. First, the effect of *Mstn* inactivation varied between genders in both models. Notable increases in absolute maximal force and power occur later in female KO^{Lee} mice as compared to male KO^{Lee} mice. Moreover, in contrast to male KO^{Grobet} mice, there was no reduction in specific maximal force, and absolute maximal force increased more in female KO^{Grobet} mice. Secondly, gender dimorphism decreased with *Mstn* inactivation in both models. In contrast to WT mice, there were no differences between sexes concerning absolute maximal force and absolute maximal power in both models. Sexual dimorphism concerning performance was reduced in KO mice, because absolute maximal force and power continued to increase beyond age 3 months in female KO^{Lee} mice, and specific maximal force was not reduced in female KO^{Grobet} mice. These results confirmed that the effects of *Mstn* inactivation on absolute and specific maximal forces can vary between sexes (7; 9).

Less is known concerning the interaction of *Mstn* and other regulators of muscle growth, such as sex hormones (14; 21–23). Recent studies reported that *Mstn* is an androgen target in skeletal muscle (24–26). We found that castration decreased the effect of *Mstn* inactivation on absolute maximal force in 6-month old male KO^{Lee} mice (there was no longer any difference between genotypes in castrated male mice). In contrast, castration increased the effect of *Mstn* inactivation on both absolute maximal force and power in 3-month old female KO^{Lee} mice (a difference appears between genotypes). Together, our findings suggest that removal of endogenous androgens and estrogens, respectively attenuates and promotes the effect of *Mstn* inactivation on some aspects of muscle performance. Thus, it would be of

interest to explore the mechanisms of this potential interaction between *Mstn* and sex hormones.

Conclusion

This study demonstrates that constitutive *Mstn* inactivation increases absolute maximal force of 6-month old KO mice in both models and sexes. Similarly, absolute maximal power is increased by genetic deficiency of myostatin at 6 months of age in both sexes, at least in the Lee model. The gain in muscle performance largely varies between models and sexes, but not for methodological reasons. Moreover, this effect of *Mstn* inactivation might not be observed in younger KO mice, at least in the Lee model. In contrast, specific maximal force is reduced with *Mstn* inactivation and occurs earlier during postnatal growth, but the extent of the reduction also varies between models and sexes. In the Lee model, reduced specific maximal force was not related to the hypertrophic effect of *Mstn* inactivation, at least in younger mice. Moreover, *Mstn* inactivation reduced the sexual dimorphism regarding absolute maximal force in both models. We show that castration modulates the effect of *Mstn* inactivation, at least in the Lee model, confirming the notion that endogenous androgens and estrogens might attenuate and promote, respectively the effect of *Mstn* inactivation on some aspects of performance. Together, these results indicate that endogenous myostatin plays an important role during postnatal skeletal muscle development in mice. It limits the gains in absolute maximal force and power, but in return it improves muscle quality, notably with regard to high intensity muscle contractile function, particularly in male mice. However, the role of endogenous myostatin varies between models and sexes, for yet unknown physiological reasons, as we excluded methodological bias.

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Abbreviations

KO : knockout, with genetic myostatin inactivation

KO^{Grobet} : mice with genetic myostatin inactivation from the model of Grobet laboratory

$KO^{Lee:}$ mice with genetic myostatin inactivation from the level of Lee laboratory

Mstn : gene encoding myostatin

P0 : absolute maximal force

Pmax : absolute maximal power

sP0 : specific maximal force

sPmax : specific maximal power

TA : *tibialis anterior* muscle

WT : wild-type

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Legends of figures

Figure 1. Absolute and specific maximal forces of tibialis anterior muscles in male (AC) and female (BD) KO^{Lee} mice. A and B : absolute maximal force (P0) ; C and D : specific maximal force (sP0). a : KO different from corresponding WT ($P < 0.05$). c : male different from corresponding female ($P < 0.05$). n=8-28/group for female mice and n=6-22/group for male mice

Figure 2. Absolute and specific maximal powers of tibialis anterior muscle in male (A,C) and female (B,D) KO^{Lee} mice. A and B : absolute maximal power (Pmax) ; C and D : specific maximal power (sPmax). a : KO different from corresponding WT ($P < 0.05$).
c : male mice different from corresponding female mice ($P < 0.05$). n=6-14/group for female and n=6-12/group for male

Figure 3. Muscle weight of tibialis anterior muscle in male (A) and female (B) KO^{Lee} mice.
a : KO different from corresponding WT ($P < 0.05$). c : male different from corresponding female ($P < 0.05$). n=8-28/group for female mice and n=6-22/group for male mice

Figure 4. Absolute and specific maximal forces, and weight of tibialis anterior muscle in male (A) and female (B) HTZ^{Lee} mice. HTZ : heterozygous mice ; P0 : absolute maximal force ; sP0 : specific maximal force. a : KO or HTZ different from corresponding WT ($P < 0.05$). d : KO different from corresponding HTZ ($P < 0.05$). n=6-34/group for female mice and n=6-28/group for male mice

Figure 5. Absolute maximal force and power, and weight of tibialis anterior muscle in

castrated male (A-C) and female (D-F) KO^{Lee} mice. P0 : absolute maximal force ; sP0 : specific maximal force ; Pmax : absolute maximal power ; sPmax : specific maximal power. a : KO different from corresponding WT ($P < 0.05$). n=7-22/group for female mice and n=8-28/group for male mice.

Figure 6. Absolute (A) and specific (B) maximal forces, and weight (C) of tibialis anterior muscle in male and female KO^{Grobet} mice. P0 : absolute maximal force ; sP0 : specific maximal force ; a : KO different from corresponding WT ($P < 0.05$); c : male different from corresponding female ($P < 0.05$); n=8-16/group for female mice and n=10-16/group for male mice

Table 1. Effect of *Mstn* inactivation in both Lee and Grobet mouse models at age 6 months.

	KO^{Lee}		KO^{Grobet}	
	Male	Female	Male	Female
Absolute maximal force (P0)	+41%	+55%	+16%	+65%
Specific maximal force (sP0)	-34%	-20%	-35%	=
Muscle weight	+114%	+96%	+75%	+87%

=: no significant difference; -: decrease; +: increase; KO^{Grobet} : mice with myostatin inactivation from the model of Grobet; KO^{Lee}: mice with myostatin inactivation from the level of Lee.

Figure 1

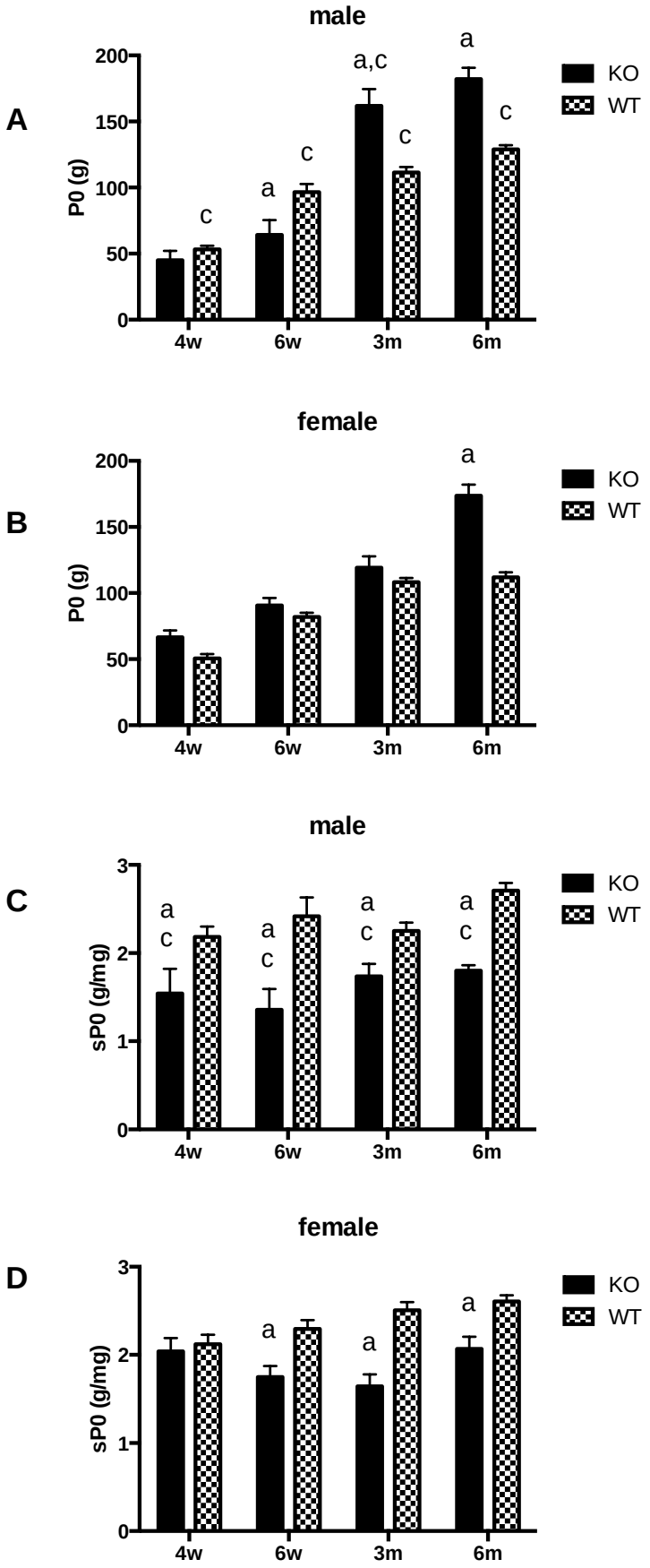

A
male
P0 (g)
200
150
100
50
0
4w
6w
3m
6m
a
c
a
c
a,c
c
a
c
KO
WT
B
female
P0 (g)
200
150
100
50
0
4w
6w
3m
6m
a
KO
WT
C
male
sP0 (g/mg)
3
2
1
0
4w
6w
3m
6m
a c
a c
a c
a c
KO
WT
D
female
sP0 (g/mg)
3
2
1
0
4w
6w
3m
6m
a
a
a
KO
WT

Figure 2

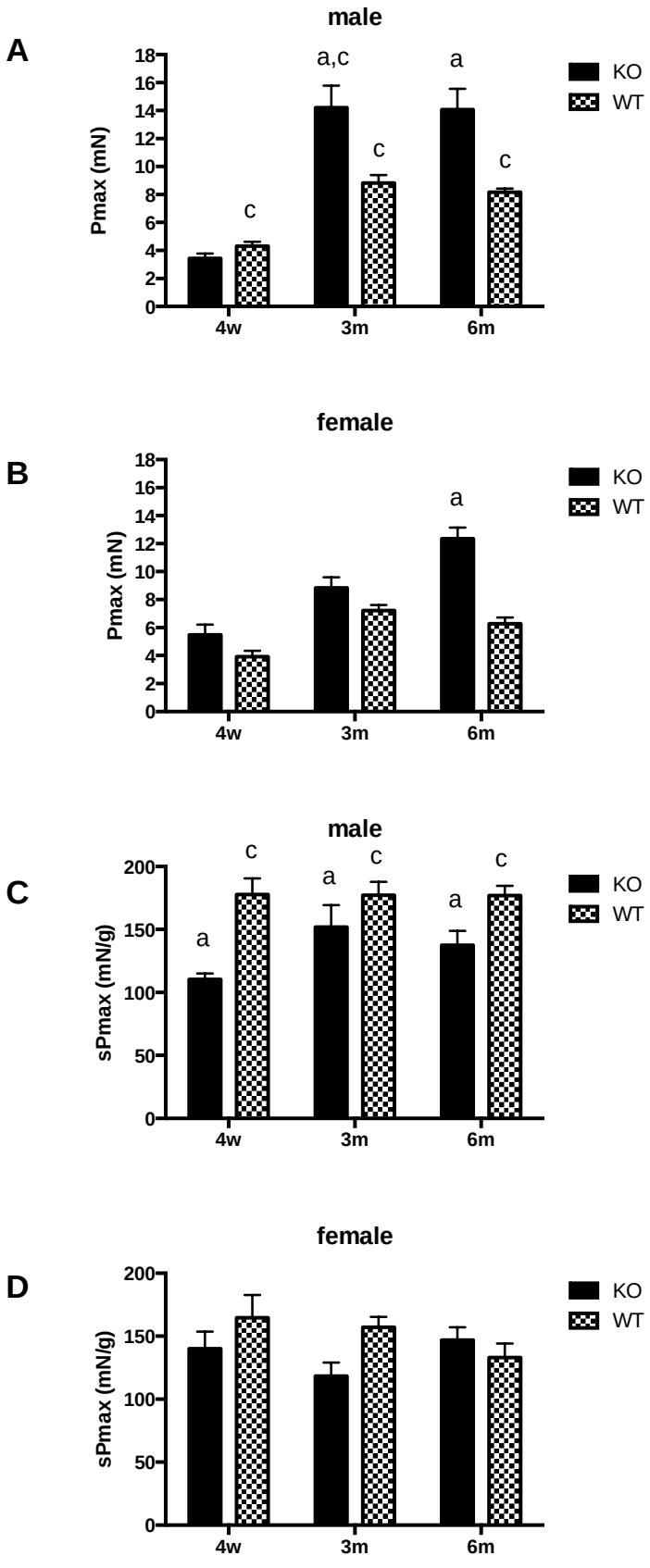
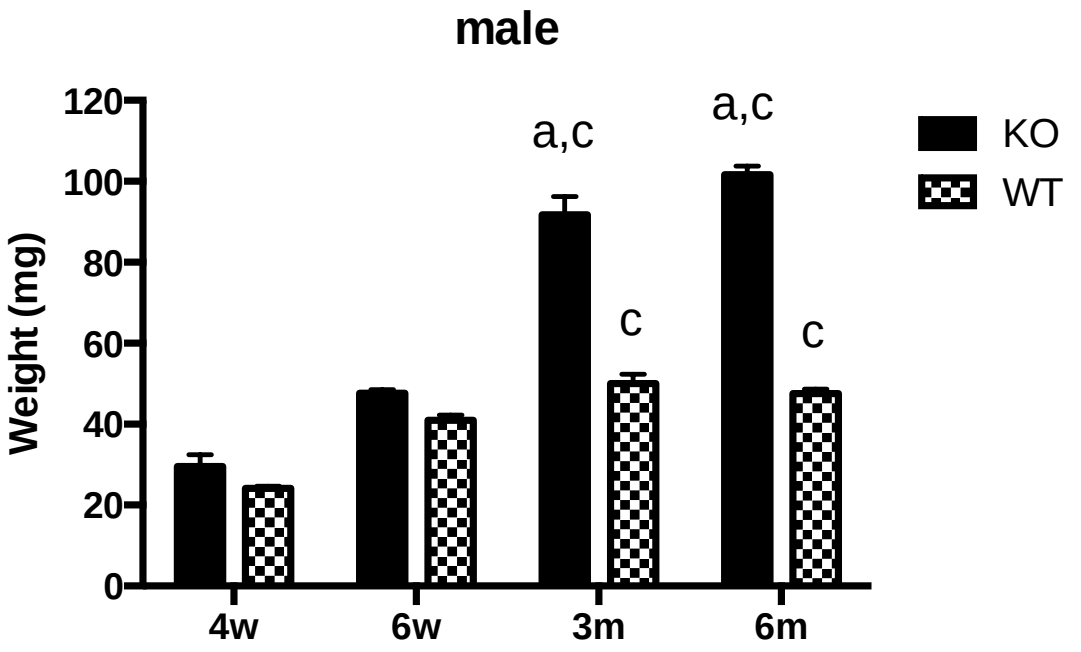

A
male
Pmax (mN)
a,c
a
c
c
c
KO
WT
4w
3m
6m
B
female
Pmax (mN)
a
KO
WT
4w
3m
6m
C
male
sPmax (mN/g)
a
c
a
c
a
c
KO
WT
4w
3m
6m
D
female
sPmax (mN/g)
KO
WT
4w
3m
6m

Figure 3

A


male
Weight (mg)
120
100
80
60
40
20
0
4w
6w
3m
6m
a,c
a,c
c
c
KO
WT

B

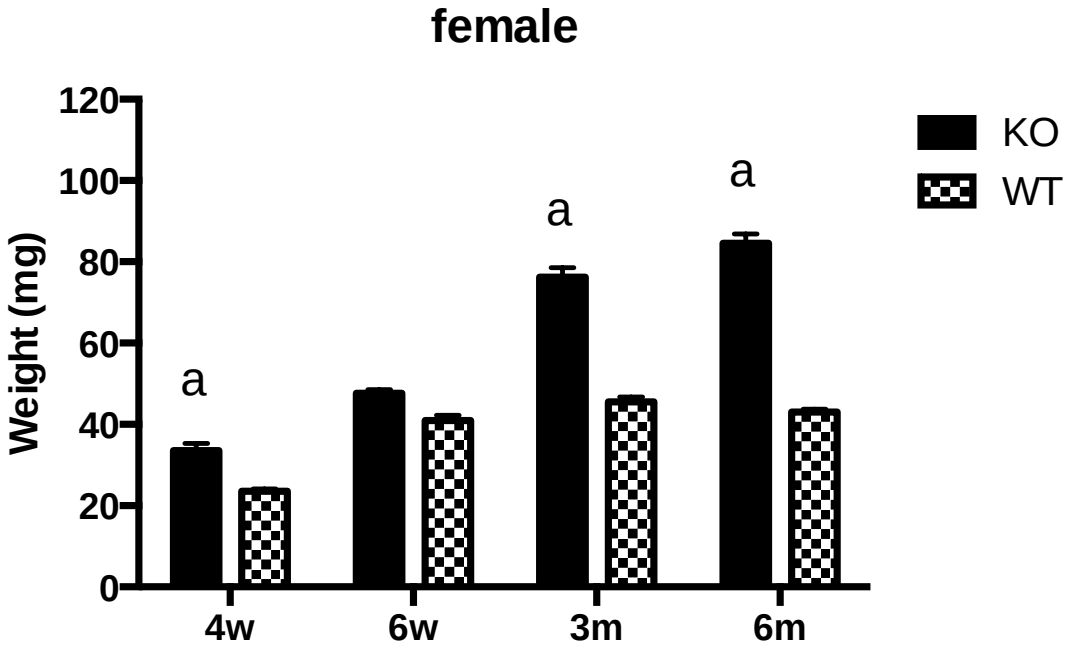
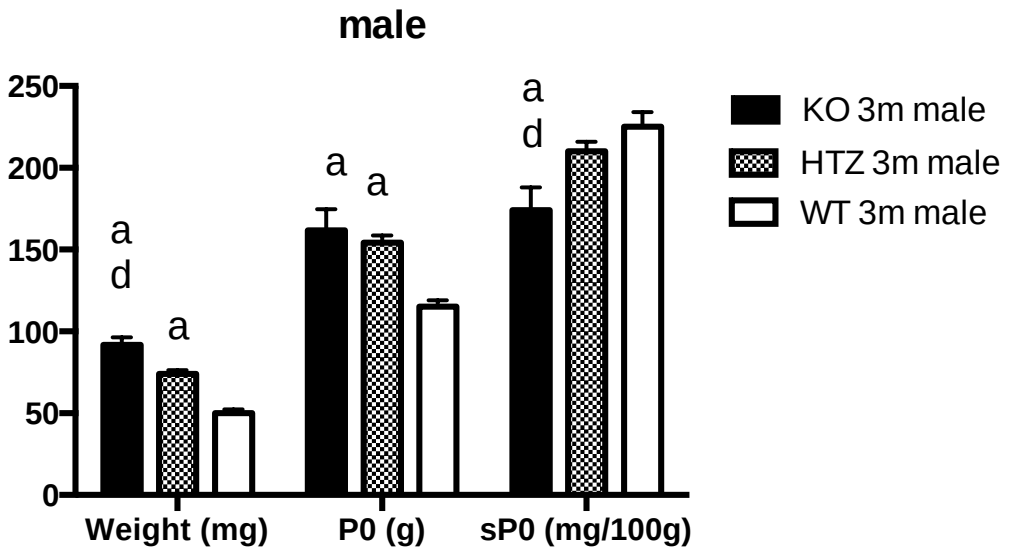

female
Weight (mg)
120
100
80
60
40
20
0
4w
6w
3m
6m
a
a
a
KO
WT

Figure 4

A


male
250
200
150
100
50
0
a
d
a
a
a
d
Weight (mg)
P0 (g)
sP0 (mg/100g)
KO 3m male
HTZ 3m male
WT 3m male

B

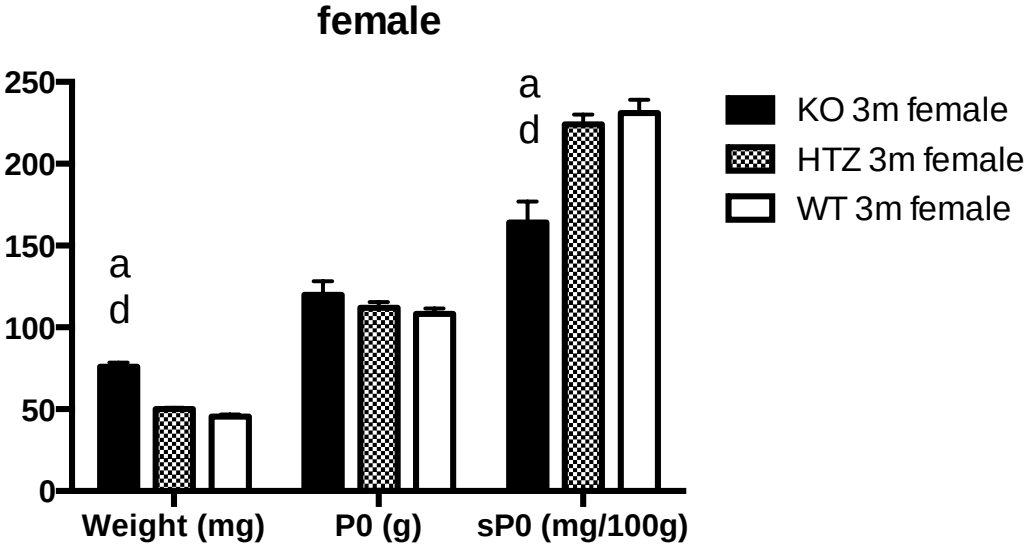

female
250
200
150
100
50
0
a
d
a
d
Weight (mg)
P0 (g)
sP0 (mg/100g)
KO 3m female
HTZ 3m female
WT 3m female

Figure 5

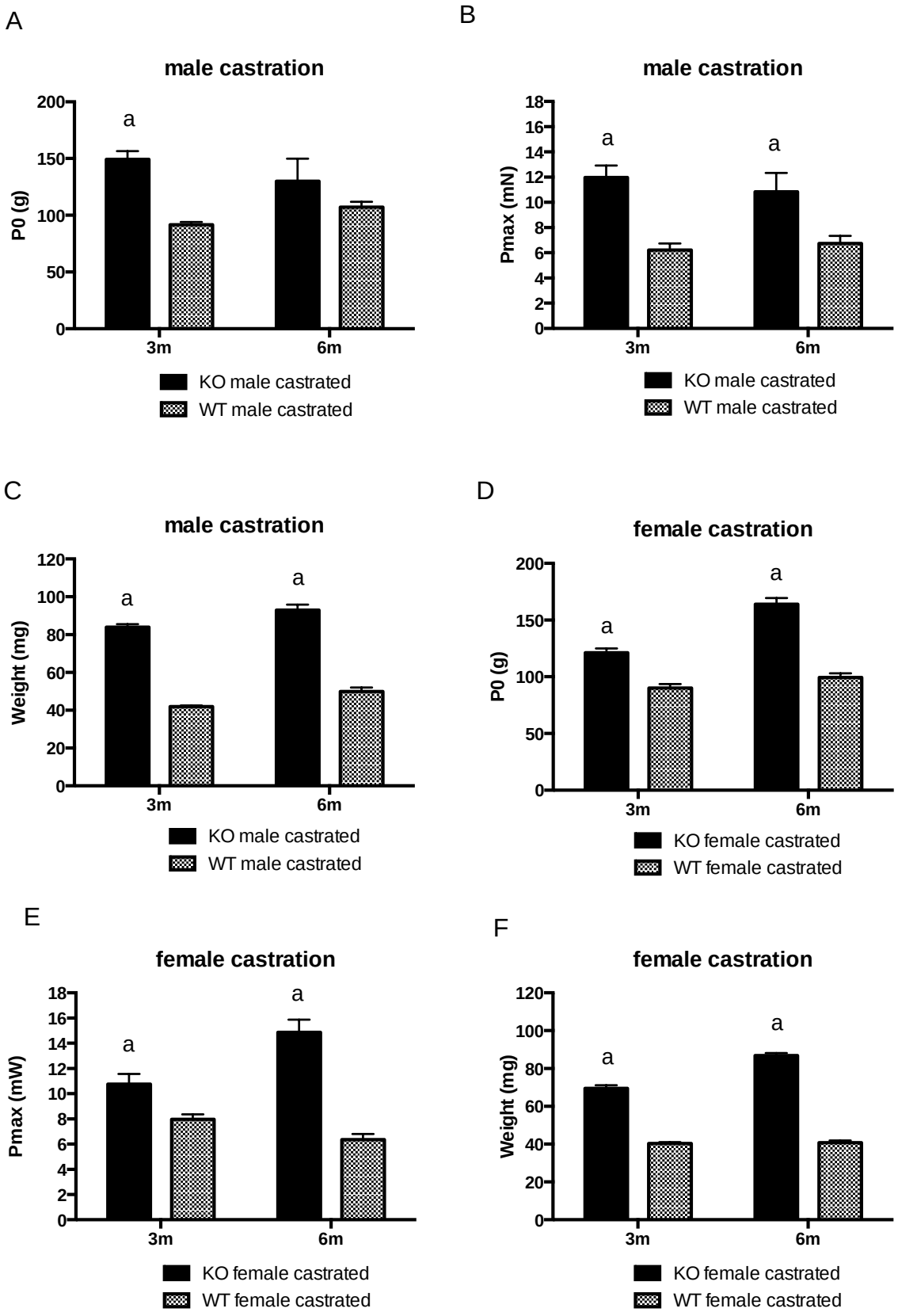
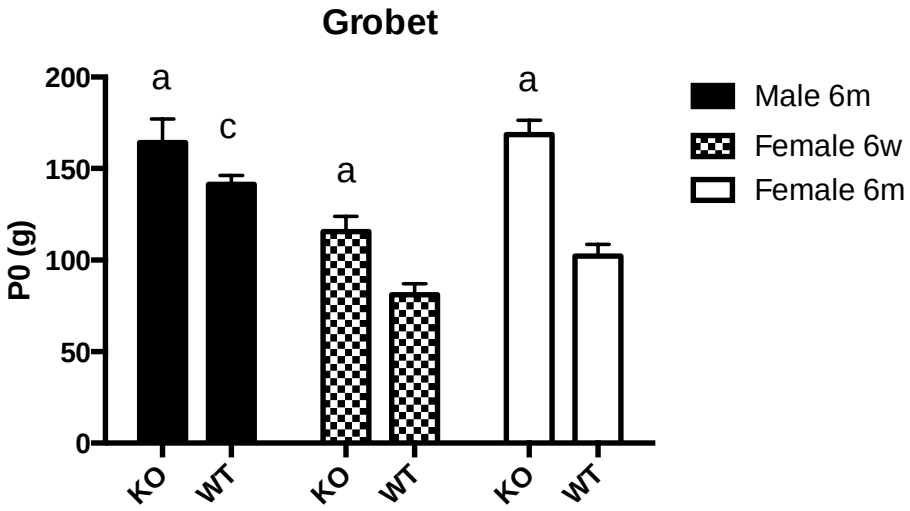
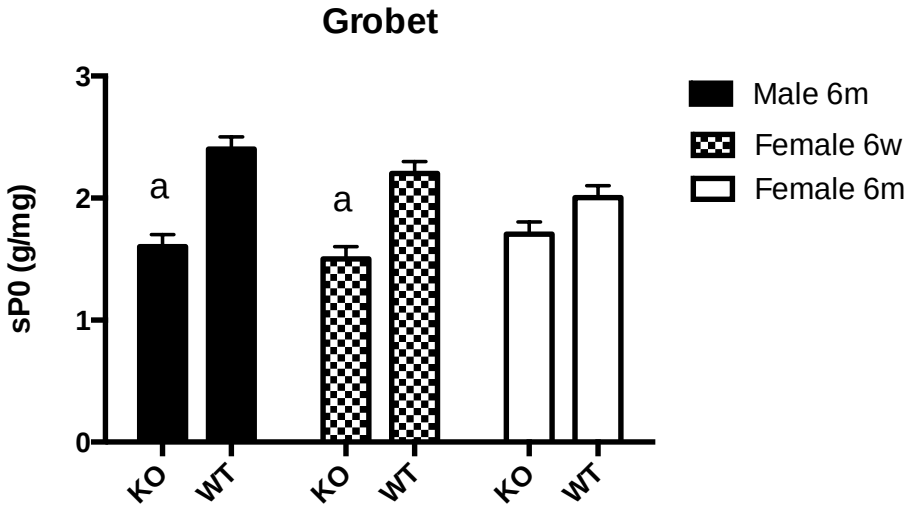

A
male castration
P0 (g)
200
150
100
50
0
a
3m
6m
KO male castrated
WT male castrated
B
male castration
Pmax (mN)
18
16
14
12
10
8
6
4
2
0
a
a
3m
6m
KO male castrated
WT male castrated
C
male castration
Weight (mg)
120
100
80
60
40
20
0
a
a
3m
6m
KO male castrated
WT male castrated
D
female castration
P0 (g)
200
150
100
50
0
a
a
3m
6m
KO female castrated
WT female castrated
E
female castration
Pmax (mW)
18
16
14
12
10
8
6
4
2
0
a
a
3m
6m
KO female castrated
WT female castrated
F
female castration
Weight (mg)
120
100
80
60
40
20
0
a
a
3m
6m
KO female castrated
WT female castrated

Figure 6

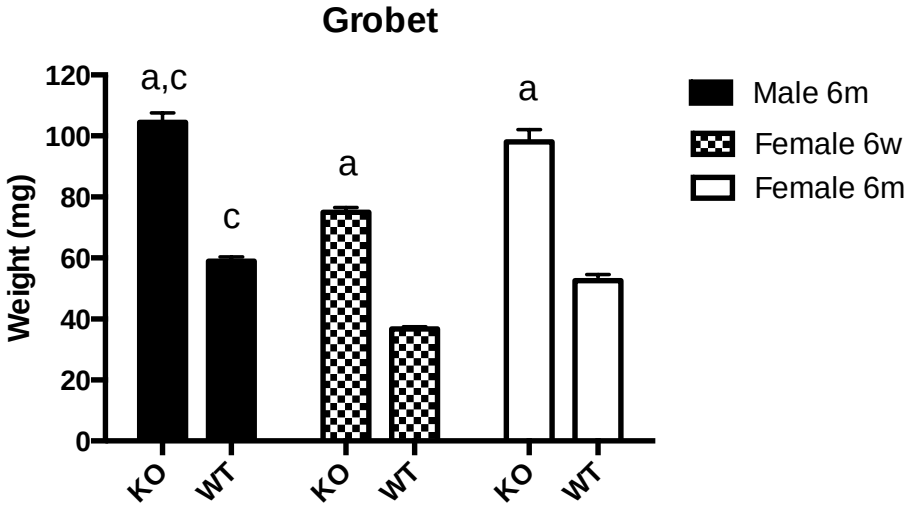
A


Grobet
P0 (g)
200
150
100
50
0
a
c
a
a
KO
WT
KO
WT
KO
WT
Male 6m
Female 6w
Female 6m

B


Grobet
sP0 (g/mg)
3
2
1
0
a
a
KO
WT
KO
WT
KO
WT
Male 6m
Female 6w
Female 6m

C


Grobet
Weight (mg)
120
100
80
60
40
20
0
a,c
c
a
a
KO
WT
KO
WT
KO
WT
Male 6m
Female 6w
Female 6m