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IGF1 does not overcome sexual dimorphism of body and muscle size in *Mstn*^{-/-} mice

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Abstract

Insulin-like growth factor-1 (IGF1) is crucial for regulating post-natal growth and, along with myostatin (MSTN), regulates muscle size. Here, we sought to clarify the roles of these two genes in regulating sexually dimorphic growth of body and muscle mass. In the first study, we established that *Igf1* mRNA was increased to a greater extent and *Igf1* receptor mRNA increased earlier in male, than in female, *gastrocnemius* muscles during the rapid phase of growth (from 2 to 6 weeks) were unchanged, thereafter, to 32 weeks of age in WT mice ($P < 0.001$). In the second study, we sought to determine if supplemental IGF1 could overcome the sexual dimorphism of muscle and body mass, when myostatin is absent. We crossed myostatin null (*Mstn*^{-/-}) mice with mice over-expressing *Igf1* in skeletal muscle (*Igf1*⁺) to generate six genotypes; control (*Mstn*^{+/+}), *Mstn*^{+/-}, *Mstn*^{-/-}, *Mstn*^{+/-}:*Igf1*⁺, *Mstn*^{-/-}:*Igf1*⁺ and *Mstn*^{-/-}:*Igf1*⁺ ($n = 8$ per genotype and sex). In both sexes, body mass at 12 weeks was increased by at least 1.6-fold and muscle mass by at least 3-fold in *Mstn*^{-/-}:*Igf1*⁺ compared with *Mstn*^{+/+} mice ($P < 0.001$). The abundance of AKT was increased in muscles of mice transgenic for *Mstn*, while phosphorylation of AKT^{S473} was increased in both male and female mice transgenic for *Igf1*⁺. The ratio of phosphorylated to total AKT was 1.9-fold greater in male mice ($P < 0.001$). Thus, despite increased growth of skeletal muscle and body size when myostatin was absent and IGF1 was in excess, sexual dimorphism persisted, an effect consistent with greater IGF1-induced activation of AKT in skeletal muscles of males.

Key Words

- ▶ skeletal muscle
- ▶ AKT
- ▶ growth
- ▶ myofibre hypertrophy
- ▶ myofibre hyperplasia
- ▶ GH
- ▶ STAT5B
- ▶ IGF1
- ▶ IGF1 receptors
- ▶ myostatin
- ▶ sexual dimorphism

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Introduction

Male mammals generally have a larger body size and muscle mass than females and, in mice, this arises from a faster rate of growth of muscle and bone before epiphyseal closure (Griffin & Goldspink 1973, Callewaert *et al.* 2010). Insulin-like growth factor-1 (IGF1) is widely recognised to be the major regulator of post-natal growth and sexually dimorphic growth (Baker *et al.* 1993, Liu *et al.* 1993, Lupu *et al.* 2001). Global deletion of IGF1 results in mice

that are 70% smaller than WT controls as adults and sexual dimorphism of body size is lost (Baker *et al.* 1993, Liu & LeRoith 1999, Lupu *et al.* 2001).

Growth hormone (GH) regulates the synthesis and secretion of IGF1. The premise is that GH acts on GH receptors to induced phosphorylation of the transcription factor STAT5B to regulate transcription of IGF1 (Le Roith *et al.* 2001). When STAT5B is absent,

sexually dimorphic growth and the difference in body and muscle mass between male and female mice was initially shown to be eliminated (Udy *et al.* 1997, Oldham *et al.* 2009). However, a subsequent study showed that sexual dimorphism persisted but with much reduced amplitude (Paul *et al.* 2019). The growth deficit is more pronounced in humans, where naturally occurring loss-of-function mutations cause retardation of growth (Kofoed *et al.* 2003, Hwa 2016). Therefore, IGF1 is thought to be crucial, not only for regulating post-natal growth but also for regulating sexually dimorphic growth.

The source of IGF1 is also important. Locally produced IGF1 (including skeletal muscle), rather than liver derived, is now understood to have the dominant influence on post-natal growth because liver-specific deletion of IGF1 had no effect on post-natal growth of mice, despite reducing circulating concentrations of IGF1 by 75% (Sjogren *et al.* 1999, Yakar *et al.* 1999). Later studies also reported that IGF1 in skeletal muscle is likely to be the major contributor to post-natal growth, because genetic removal of *Stat5a* and *5b* from skeletal muscle reduced post-natal growth of mice (Klover & Hennighausen 2007, Klover *et al.* 2009). In the same studies, no further loss of growth was observed when *Stat5b* was also eliminated from the liver (Klover & Hennighausen 2007, Klover *et al.* 2009). While local expression of IGF1 is the predominant regulator of post-natal growth, circulating IGF1, derived from liver, accounts for about 30% of post-natal growth and adult body size (Stratikopoulos *et al.* 2008).

Myostatin, too, has a profound, but inhibitory, influence on the growth of skeletal muscle during embryogenesis and post-natal growth. Myostatin is present in skeletal muscle at the earliest stages of muscle development and inhibits myogenesis. In the absence of myostatin, mice have an increased number of myofibres (McPherron *et al.* 1997, Lee & McPherron 1999) and an unaffected (Hennebry *et al.* 2017), or increased (McPherron *et al.* 1997, Amthor *et al.* 2007), size of muscle fibres depending on a given muscle. In contrast to the action on muscle, myostatin directs the fate of progenitor cells towards a lipogenic lineage (Artaza *et al.* 2005), thereby having a profound effect on body composition.

We and others have shown that myostatin protein is reduced in skeletal muscles of adult male mice (McMahon *et al.* 2003, Reisz-Porszasz *et al.* 2003) and that the reduction is regulated by GH acting on STAT5B (Oldham *et al.* 2009). We also reported that the post-translational decrease in mature myostatin is initiated between 6 and 12 weeks of age in males and coincides with the onset of sexually dimorphic growth. From 6 weeks,

the divergence in growth and abundance of mature myostatin protein between the sexes were more apparent, reaching the greatest difference when mice were 32 weeks of age. Therefore, we proposed that myostatin not only regulates the growth of skeletal muscles in post-natal life but also regulates sexually dimorphic growth and maintenance of the mass of adult skeletal muscles.

Myostatin and IGF1 have opposing actions on common and independent signal transduction pathways. Myostatin binds and activates activin type 2B receptors to promote phosphorylation of SMAD2/3 (Rebbapragada *et al.* 2003) and inhibits RPS6KB1 and RPS6 to regulate protein synthesis (Trendelenburg *et al.* 2009, Welle *et al.* 2009). In contrast, IGF1 activates the IGF1 receptor and phosphorylates PI3 kinase (PI3K) and AKT (Rommel *et al.* 2001). The PI3K/AKT pathway is common to both factors and there is considerable cross-regulation between the two, wherein myostatin inhibits AKT to block IGF1-induced hypertrophy of myotubes (Morissette *et al.* 2009), IGF1 inhibits myostatin-induced activation of SMAD3 (Retamales *et al.* 2015). Thus, the two factors, having opposing actions, could act in an additive, or synergistic manner via common, or distinct pathways to establish muscle and inhibit fat mass.

We have recently confirmed that both IGF1 and myostatin interact to regulate post-natal growth of body and muscle mass in male mice (Hennebry *et al.* 2017). In preliminary observations, we noticed that there was less *Igf1Ea* mRNA (the principal endogenous *Igf1* transcript) in *quadriceps* muscles of *Mstn*^{-/-} mice at 4 and 8 weeks of age (about half the amount) compared with WT (*Mstn*^{+/+}) mice. Furthermore, there were lower concentrations of *Igf1Ea* mRNA after 8 weeks of age reaching similar concentrations by 20 weeks in both strains (Hennebry *et al.* 2017). Therefore, we proposed that rates of growth, body and muscle mass would be increased if IGF1 protein were increased in *Mstn*^{-/-} mice. Indeed, sequential removal of copies of the myostatin gene increased the number of myofibres and the addition of transgenic IGF1 in skeletal muscle increased the size of those myofibres, providing a synergistic increase in muscle mass, a reduction in fat mass and a shift in myofibre phenotype towards faster-twitch (Hennebry *et al.* 2017). We further showed that loss of myostatin increased the abundance of the cell signalling intermediate AKT, while additional IGF1 increased the phosphorylation of AKT. Therefore, both IGF1 and myostatin interact to regulate the signalling of AKT.

In the current study, we sought to determine the effect of the interaction of IGF1 and myostatin on AKT

signalling and sexually dimorphic growth. We first examined the expression of *Igf1Ea* and *Igf1r* receptors (*Igf1r*) in skeletal muscles and concentrations of IGF1 in blood during post-natal growth of male and female WT mice to determine the pattern of expression in post-natal muscle. In addition, we hypothesised that in the absence of myostatin and with IGF1 in excess, the rates of growth would be greater in both sexes and sexual dimorphism of body and muscle mass might be reduced, or abolished.

Materials and methods

Animals

Study 1 Quantification of *Igf1Ea* and *Igf1r* mRNA in muscles of WT mice during post-natal growth from 2 to 32 weeks of age

Male and female mice of the C57BL/6J strain were killed at 2, 3, 4, 6, 12, 20 and 32 weeks of age ($n = 6$ of each sex per age).

Study 2 Development of mice transgenic for *Igf1* in skeletal muscles and myostatin

Generation of *Mstn*^{-/-} mice (where all tissues lack expression of *Mstn*) has been previously described (McPherron *et al.* 1997). We obtained a breeding pair of these mice as a gift from S-J Lee (Johns Hopkins University School of Medicine, Baltimore, MD, USA).

Transgenic *Igf1* (C2:Ea) mice were generated at the European Molecular Biology Laboratory, Rome, Italy by N Winn and N Rosenthal. The MLC/*Igf1* (C2:Ea) expression construct was generated by cloning the mouse *Igf1* (C2:Ea) cDNA sequences into the previously described skeletal muscle-specific expression cassette containing the myosin light chain (MLC) 1/3 promoter, a SV40 polyadenylation signal, followed by the MLC1/3 enhancer sequence (Rosenthal *et al.* 1989, Musaro *et al.* 2001, Shavlakadze *et al.* 2010). In these MLC/*Igf1* transgenic mice, IGF1 is most highly expressed in fast-twitch myofibres in the order 2B, 2X and 2A myosin heavy chain (MyHC) (Shavlakadze *et al.* 2005).

The *Igf1* (C2:Ea) (FVB background) and *Mstn*^{-/-} (C57BL/6J background) mice were maintained as separate colonies at the Ruakura Research Centre, Hamilton, New Zealand. The F1 generation from a cross of *Igf1* (C2:Ea) and *Mstn*^{-/-} founders was used to produce six genotypes in the F2 offspring: *Mstn*^{+/+} (no *Igf1* transgene), *Mstn*^{+/-}, *Mstn*^{-/-}, *Mstn*^{+/+}:*Igf1*⁺, *Mstn*^{+/-}:*Igf1*⁺ and *Mstn*^{-/-}:*Igf1*⁺ (*Igf1*⁺ refers to the *Igf1* (C2:Ea) transgene).

For both studies, mice were housed 2–4 per cage in a temperature controlled (22°C) and light controlled (12 h light:12 h darkness cycle) animal facility at Ruakura Research Centre, Hamilton, New Zealand. Mice had access to a standard rodent chow (Specialty Feeds, Glen Forrest, WA 6071, Australia) and water *ad libitum*.

The studies were approved by the Ruakura Animal Ethics Committee.

Phenotype analyses of six genotypes during early post-natal growth (sampled at 12 weeks of age) (study 2 only)

Male ($n = 48$) and female ($n = 48$) pups with or without the *Igf1* (C2:Ea) transgene that had a matching myostatin genotype (*Mstn*^{+/+}, *Mstn*^{+/-}, or *Mstn*^{-/-}) within litters were included in the study ($n = 8$ per genotype and sex). Mice were weighed weekly and killed at 12 weeks of age and many tissues were sampled for molecular analyses as outlined below.

Muscle and fat pad collection (studies 1 and 2)

Mice were not fasted before death and were killed using CO₂ asphyxiation followed by cervical dislocation. At death, a sample of blood was collected via cardiac puncture into a tube containing EDTA. Plasma was harvested and stored at -20°C before the assay of IGF1.

Following euthanasia, the *Quadriceps femoris* (*quadriceps*), *B. femoris*, *gastrocnemius*, *plantaris*, *soleus*, *Extensor digitorum longus* (EDL), *Tibialis anterior* (*T. anterior*) muscles were excised from both hind limbs, along with the heart, which was blotted to remove excess blood, and weighed. The *soleus* is a slow-twitch muscle. The *T. anterior*, *plantaris* and *EDL* are fast-twitch muscle and the *quadriceps*, *B. femoris* and *gastrocnemius* muscles have a mixed myofibre composition (Hamalainen & Pette 1993, Augusto *et al.* 2004). Because mouse muscles are small and some were used in a previous study (Hennebry *et al.* 2017), we used the *gastrocnemius* for qPCR in study 1 and the *quadriceps* muscle for qPCR, Western analyses and myofibre typing for study 2.

The length of the tibia was measured and used to normalise the mean masses of skeletal muscles (White *et al.* 2016) and the heart was normalised to body mass (Hagdorn *et al.* 2019). The gonadal fat pads were excised and weighed and were expressed as a percent of body mass. The gonadal fat pads correlate highly with total body fat (Rogers & Webb 1980).

Table 1 Primers used for qPCR (studies 1 and 2).

Gene	Primer sequences	Size (bp)	Annealing (°C)	GenBank Accession
<i>Igf1Ea</i>	Fwd: GCTTGCTCACCTTTACCAGC Rev: AAATGTACTTCCTTCTGGGTCT	301	60	NM_001111275.2
<i>Igf1</i> (C2:Ea)*	Fwd: ACTGACATGCCCAAGACTCAG Rev: ATTCCACCACTGCTCCCATTC	283	60	Shavlakadze <i>et al.</i> 2010
<i>Igf1r</i>	Fwd: CTGCGGCGATGAAGAGAAGAAAA Rev: ACCGGTGCCACGTTATGATGATT	546	60	NM_010513.2

*Fwd primer is common to exon 4, while the reverse primer is for the SV40 enhancer of the transgene.

RNA extraction and quantitative (q) PCR (studies 1 and 2)

Total RNA was extracted from the *gastrocnemius* (study 1) and from the *quadriceps* muscles (study 2) using Trizol reagent (Invitrogen). cDNA was reverse transcribed (RT) from 2 µg total RNA using qSCRIPT (Quanta BioSciences, Maryland, USA) as described previously (Smith *et al.* 2014). In study 1, qPCR was carried out using a Lightcycler 2.0 (Roche Diagnostics) and Roche Faststart DNA master PLUS SYBR Green I mix (Roche Diagnostics). In study 2, qPCR was carried out using a RotorGene 6000 (Corbett Research, Bio-Strategy Limited). The primers for *Igf1Ea*, *Igf1* (C2:Ea) and *Igf1* receptor are presented in Table 1. Standard curves were established by serial dilution of a pool of cDNA obtained from each sample and results were normalised to the concentration of ssDNA in the RT samples (Lundby *et al.* 2005).

Western blot analyses (study 2 only)

Protein was extracted from the *quadriceps* muscle and Western blotting was performed as previously described (Smith *et al.* 2014). Membranes were incubated with rabbit anti-AKT1/2/3 (1:3000, #8312, Santa Cruz Biotechnology, Inc.), rabbit anti-phospho-AKT1^{S473} monoclonal (1:10,000, #44-621G, Invitrogen) and rabbit monoclonal anti-GAPDH (1:10,000, #EPR16891, Abcam) and the abundance of each protein was detected with enhanced chemiluminescence. GAPDH was used as a loading control. The images were captured with a ChemiDoc MP Imaging System (Bio-Rad Laboratories (NZ) Pty Ltd) and the optical densities of each immunoreactive band were obtained using Image Lab software (Bio-Rad Laboratories (NZ) Pty Ltd).

Assessment of myosin heavy chain isoforms (MyHC) (study 2 only)

Changes in the composition of MyHC in quadriceps muscles were determined electrophoretically as described

previously (Smith *et al.* 2014). Despite improvements in separation of the bands using an 8 M urea/2 M thiourea buffer in which the samples were loaded, the 2A and 2X bands typically do not separate as cleanly in mouse skeletal muscle as they do in rat skeletal muscle (Blough *et al.* 1996). Therefore, we measured MyHC 2A and 2X as one band. A 50:50 mixture of mouse *soleus* and *EDL* muscle were run in the gels to act as a marker to help identify MyHC bands.

IGF1 assay (Studies 1 and 2)

Concentrations of IGF1 protein were assayed in plasma in both studies and in homogenates from *quadriceps* muscles in study 2 using a mouse/rat IGF1 ELISA kit (#MG100, Quantikine ELISA, R&D Systems Inc). Concentrations of IGF1 were normalised to the concentration of total protein (measured by BCA assay) in the homogenate of *quadriceps* muscles.

Statistical analysis

All data were analysed with Prism version 8.3.0 for Windows, GraphPad Software. In study 1, two-way ANOVA was used with sex and time as the treatment terms. *Post hoc* analyses were made with Fisher's Uncorrected LSD test because multiple comparison among means were not required. In study 2, three-way ANOVA was used with IGF1, MSTN and Sex as the treatment terms. *Post hoc* analyses were made with Tukey's multiple comparison test and with Fisher's Uncorrected LSD test when multiple comparisons were and were not required, respectively. Data are presented as means and the S.E.M.

Results

Study 1

Rate of growth

In study 1, body mass (Fig. 1A) and the mass of *gastrocnemius* muscles (Fig. 1B) increased equally in both sexes until

Figure 1

Body and muscle mass and rates of growth dynamics in WT mice during post-natal growth (study 1). (A) body mass (mean $\pm$ s.e.m.), (B) *gastrocnemius* mass, (C) growth rate of body mass and (D) growth rate of *gastrocnemius* mass in male and female mice of the C57 strain from 2 to 32 weeks of age ($n = 6$ per group). Asterisks indicate significance between sex at each time point (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

4 weeks of age, after which there was a precipitous decline in the rates of growth of body and muscle mass in both sexes, with a greater reduction in females than in males (Fig. 1C and D).

Expression of *Igf1Ea* and *Igf1r* mRNA

Igf1Ea mRNA increased in the *gastrocnemius* muscles of both sexes but to a greater extent (about two-fold) in muscles of males, than females, with peak expression observed at 6 weeks of age, before declining in both sexes to 32 weeks of age (Fig. 2A). In contrast, *Igf1r* mRNA increased equally in the *gastrocnemius* muscles of both sexes but peaked earlier at 4 weeks in males and later at 6 weeks in females (Fig. 2B).

Concentrations of IGF1 protein in plasma

Concentrations of IGF1 protein in plasma were not significantly different across time or between male and female mice. However, a bimodal pattern of was evident in blood, wherein concentrations were increased between 3 and 4 weeks and again at 20 weeks. The first peak coincided with the increased rate of body and muscle mass (Fig. 3).

Study 2

Expression of transgenic *Igf1Ea* mRNA

We confirmed that the *Igf1* transgene was expressed in *quadriceps* muscles of the *Mstn*^{+/+}*Igf1*⁺, *Mstn*^{+/-}*Igf1*⁺ and *Mstn*^{-/-}*Igf1*⁺ mice and absent in those of *Mstn*^{+/+}, *Mstn*^{+/-} and *Mstn*^{-/-} mice (Fig. 4A). In addition, we observed that expression of the *Igf1* transgene was two- to four-fold higher in *quadriceps* muscles of males, than females

($P < 0.001$). Furthermore, expression of the *Igf1* transgene increased in males, but not females, as copy number of the myostatin gene decreased (MSTN $\times$ Sex $\times$ IGF1 interaction, $P < 0.01$) (Fig. 4A). Similarly, concentrations of IGF1 protein were increased in *quadriceps* muscles of mice transgenic for *Igf1* (main effect of IGF1, $P < 0.001$). However, female *quadriceps* muscles had 1.4-fold more

Figure 2

Concentrations of mRNA in *gastrocnemius* muscles of WT mice during post-natal growth (study 1). Arbitrary concentrations (mean $\pm$ s.e.m.) of (A) *Igf1Ea* mRNA and (B) *Igf1* receptor mRNA in *gastrocnemius* muscles of male and female C57 mice from 2 to 32 weeks of age as determined by qPCR ($n = 6$ per group). Asterisks indicate significance between sex at each time point (* $P < 0.05$, *** $P < 0.001$).

Figure 3

Concentrations of IGF1 (mean $\pm$ S.E.M.) in plasma of male and female WT mice from 2 to 32 weeks of age as determined by ELISA ($n = 6$ per group) (study 1).

IGF1 protein across all genotypes than males (Sex $\times$ IGF1 interaction, $P < 0.01$) (Fig. 4B).

Phenotype of mice transgenic for IGF1 and absence of myostatin

Sequential removal of myostatin together with over-expression of *Igf1* mRNA increased the rate of growth of both sexes with an increase of ~three-fold from the *Mstn*^{+/+}

Figure 4

Concentrations of (A) *Igf1Ea* mRNA and (B) IGF1 protein in *quadriceps* muscles of *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{-/-};*Igf1*⁺ (*M*^{-/-};*I*⁺), *Mstn*^{-/-};*Igf1*⁺ (*M*^{-/-};*I*⁺) and *Mstn*^{-/-};*Igf1*⁺ (*M*^{-/-};*I*⁺) mice at 12 weeks (study 2) ($n = 8$ per genotype and sex). The mean concentration for each group is represented by the horizontal bar. Asterisks indicate significance between sex within each genotype (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

to the *Mstn*^{-/-};*Igf1*⁺ genotype ($P < 0.001$) (Fig. 5A). The main effects of MSTN, Sex and IGF1 accounted for 48.6% ($P < 0.001$), 20.4% ($P < 0.001$) and 12.7% ($P < 0.001$) of the variation, respectively. In addition, the rate of growth was 1.6-fold faster in males compared with females as copies of *Mstn* were removed and this interaction accounted for a further 2% of the variation (MSTN $\times$ Sex interaction, $P < 0.01$). Furthermore, the rate of growth was 1.4-fold faster in mice transgenic for *Igf1* as copies of *Mstn* were removed, which accounted for a further 6.6% of the variation (MSTN $\times$ IGF1 interaction, $P < 0.001$).

Similarly, body mass increased by at least 1.6-fold overall in both sexes from the *Mstn*^{+/+} to the *Mstn*^{-/-};*Igf1*⁺ genotype ($P < 0.001$), with MSTN, Sex and IGF1 accounting for 39.5% ($P < 0.001$), 24.9% ($P < 0.001$) and 13.8% ($P < 0.001$) of the variation, respectively (Fig. 5B). The *Igf1* transgene increased body mass by 1.2-fold irrespective of sex as copies of *Mstn* were removed and accounted for a further 2.6% of the variation (MSTN $\times$ IGF1 interaction, $P < 0.01$).

Females were shorter than males by ~5% overall (main effect of Sex, $P < 0.001$). In addition, there was a small (2%) increase in body length (nose to anus) attributed to the absence of *Mstn* ($P < 0.05$) but no difference among genotypes for length of the tibia (Table 2). Therefore, the increase in body mass could be largely attributed to greater growth of skeletal muscles (Fig. 5C and Table 2). The mass of *quadriceps* (Fig. 5C) as well as the *gastrocnemius*, *B. femoris*, *T. anterior*, *EDL* and *plantaris* muscles at 12 weeks of age (corrected to tibia length) were increased by up to three-fold in male *Mstn*^{-/-};*Igf1*⁺ mice and up to 3.5-fold in female *Mstn*^{-/-};*Igf1*⁺ mice (Table 2). The uncorrected mass of the hearts was not different among genotypes but was significantly lower in female, than in male mice ($P < 0.001$). Conversely, the mass of the heart corrected to body mass was not different between sexes but was reduced in the absence of myostatin ($P < 0.001$) and in mice transgenic for *Igf1* ($P < 0.001$) (Table 2). The mass of the gonadal fat pad was reduced in both sexes by 58% as copies of *Mstn* were reduced, which accounted for 17.5% of the variation (Fig. 5D, $P < 0.001$). In addition, mice transgenic for *Igf1* had 34% less gonadal fat than mice without the transgene (6.9% of the variation, $P < 0.01$).

Despite these dramatic increases in growth rate and body size, sexual dimorphism was not abolished and the differences remained remarkably constant across the genotypes, with males having a body mass that was ~25% larger ($P < 0.001$) than females within each genotype (Fig. 5B). Similarly, mass of the *quadriceps* was

Figure 5

Rates of growth, body, muscle and fat mass of WT and transgenic mice (study 2). (A) Average rate of growth, (B) Body mass, (C) Mass of the *quadriceps* muscle normalised to tibia length and (D) mass of the gonadal fat pad normalised to body mass in *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{+/-} (*M*^{+/-}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{+/-};*Igf1*⁺ (*M*^{+/-};*I*⁺), *Mstn*^{-/-};*Igf1*⁺ (*M*^{-/-};*I*⁺) and *Mstn*^{-/-};*Igf1*⁺ (*M*^{-/-};*I*⁺) mice at 12 weeks of age (*n* = 8 per genotype and sex). The mean concentration for each group is represented by the horizontal bar. Unlike letters denote significant differences between genotype (at least *P* < 0.05). Asterisks indicate significance between sex within genotype (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

on average 34% larger in males than in females (*P* < 0.001) within each genotype (Fig. 5C). A similar pattern was evident for other muscles composed of fast- or mixed-twitch myofibres (Table 2). Overall, the absence of *Mstn* had the greatest effect on muscle mass (accounting for 64% of the variance on average), followed by the *Igf1* transgene (15.4% of the variance) and then sex (7.8% of the variance) for *quadriceps*, *gastrocnemius*, *B. femoris*, *T. anterior*, *EDL* and *plantaris* muscles. The effect of the *Igf1* transgene in conjunction with absence of *Mstn* (*MSTN* × *IGF1* interaction) accounted for 7.9% of the variance, while the interaction of *MSTN* × Sex (*gastrocnemius* and *plantaris*) and *IGF1* × Sex (*T. anterior*) accounted for 1.3% and 0.9% of the variance (Table 2).

The mass of the *soleus* was also increased, although to a lesser degree (up to two-fold increase) than muscles expressing the fast 2X and 2B myosin heavy chains (Table 2). However, the mass of the *soleus* remained ~30% larger in males than in females of all genotypes.

Myofibre composition

There was a main effect of sex (*P* < 0.001) on myofibre composition, wherein males had more MyHC type 2B and less type 2A/X (these bands did not separate during electrophoresis) and type I than female *quadriceps* muscles. In addition, there was a main effect of myostatin (*P* < 0.001) wherein deletion of myostatin biased the myofibre type composition in the *quadriceps* towards 2B MyHC (9% more in males and 11% more in females, *P* < 0.001, Fig. 6A and C). By contrast, the abundance of type 2A/X MyHC in *quadriceps* was reduced by 70% in male and 65% in female *Mstn*^{-/-};*Igf1*⁺ compared with

Mstn^{+/+} controls (Fig. 6B, *P* < 0.001), with a similar pattern occurring with type 1 MyHC (Fig. 6D, *P* < 0.001).

Concentrations of IGF1 protein in plasma and *Igf1r* mRNA in *quadriceps*

We measured concentrations of IGF1 protein in blood to determine if IGF1 was getting into circulation to affect other tissues. Concentrations were increased ~1.4-fold) in plasma of male mice transgenic for *Igf1* (Sex × *IGF1* interaction, *P* < 0.001) but not different between sexes in the other genotypes (Fig. 7A).

Concentrations of *Igf1* receptor mRNA were quite variable within sex and genotype. However, there was a *MSTN* × Sex × *IGF1* interaction (*P* < 0.001), wherein concentrations were increased in female *quadriceps* muscles of *Mstn*^{+/+}, *Mstn*^{+/-};*Igf1*⁺, *Mstn*^{-/-} and reduced in muscles of both sexes of *Mstn*^{-/-};*Igf1*⁺ mice (Fig. 7B).

Abundance of tAKT and pAKT

The abundance of total (t) AKT increased in *quadriceps* muscles of male and female mice as copies of *Mstn* were removed to a maximum of 1.7- and 1.9-fold, respectively, from the *Mstn*^{+/+} to the *Mstn*^{-/-};*Igf1*⁺ genotype (main effect of *MSTN*, *P* < 0.05, Figs 8 and 9A). Furthermore, the abundance of tAKT tended to be 1.25-fold greater (*P* < 0.1) in *quadriceps* muscles of female than male mice. In contrast, the abundance of phosphorylated (p) AKT was increased in mice of both sexes transgenic for *Igf1* (*P* < 0.001) (Figs 8 and 9B). Overall, however, the ratio of p-t AKT was 1.9-fold greater (main effect of Sex, *P* < 0.001) in *quadriceps* muscles of male than in female mice (Figs 8 and 9C).

Table 2 Mass (mg) of six different skeletal muscles corrected to the length of the tibia and of the tibia length, nose to anus length (NA) and heart mass uncorrected and corrected to body mass for male and female mice of six genotypes that differed in (systemic) deletion of *Mstn* and/or addition of the *Igf1* transgene expressed specifically in skeletal muscle throughout development (sampled at 12 weeks) ($n = 8$ per genotype and sex).

Tissue	Sex	<i>Mstn</i> ^{+/+}	<i>Mstn</i> ^{+/+} ; <i>Igf1</i> ⁺	<i>Mstn</i> ^{+/−}	<i>Mstn</i> ^{+/−} ; <i>Igf1</i> ⁺	<i>Mstn</i> ^{−/−}	<i>Mstn</i> ^{−/−} ; <i>Igf1</i> ⁺	<i>P</i>						
								<i>M</i>	<i>I</i>	<i>S</i>	<i>M</i> × <i>S</i>	<i>M</i> × <i>I</i>	<i>I</i> × <i>S</i>	<i>M</i> × <i>I</i> × <i>S</i>
Gast (mg/mm)	M	7.2 ± 0.2 ^a	8.9 ± 0.3 ^b	8.5 ± 0.1 ^b	10.6 ± 0.3 ^{b,c}	14.3 ± 0.5 ^d	20.7 ± 0.8 ^e	0.001	0.001	0.001	0.001	0.001	0.339	0.869
B. fem (mg/mm)	F	5.5 ± 0.1 ^{a,***}	6.9 ± 0.2 ^{a,***}	6.7 ± 0.2 ^{a,b,***}	8.2 ± 0.4 ^{b,***}	10.4 ± 0.3 ^{c,***}	16.6 ± 0.4 ^{d,***}							
	M	9.6 ± 0.6 ^a	11.7 ± 0.4 ^a	10.9 ± 0.2 ^a	14.9 ± 0.6 ^b	17.4 ± 1.0 ^b	28.2 ± 1.5 ^c	0.001	0.001	0.001	0.063	0.001	0.556	0.796
T. ant (mg/mm)	F	7.0 ± 0.4 ^{a,***}	9.3 ± 0.5 ^{a,*}	8.6 ± 0.2 ^{a,*}	11.7 ± 0.5 ^{b,***}	13.2 ± 0.7 ^{b,***}	23.1 ± 1.0 ^{c,***}							
	M	2.8 ± 0.1 ^a	3.5 ± 0.1 ^a	3.2 ± 0.1 ^{a,b}	4.5 ± 0.2 ^b	4.9 ± 0.3 ^b	7.6 ± 0.4 ^c	0.001	0.001	0.001	0.265	0.001	0.004	0.113
EDL (mg/mm)	F	2.3 ± 0.1 ^{a,*}	2.9 ± 0.1 ^{a,***}	2.7 ± 0.1 ^{a,b,*}	3.4 ± 0.1 ^{b,***}	4.4 ± 0.2 ^{c†}	6.1 ± 0.2 ^{d,***}							
	M	0.64 ± 0.13 ^a	0.83 ± 0.14 ^a	0.66 ± 0.02 ^a	0.89 ± 0.04 ^{a,b}	1.08 ± 0.06 ^b	1.75 ± 0.05 ^c	0.001	0.001	0.001	0.565	0.001	0.559	0.732
Plantaris (mg/mm)	F	0.43 ± 0.02 ^{a,*}	0.56 ± 0.01 ^{a,***}	0.55 ± 0.02 ^a	0.68 ± 0.05 ^{a,b,*}	0.80 ± 0.05 ^{b,***}	1.52 ± 0.06 ^{c,*}							
	M	0.94 ± 0.04 ^a	1.17 ± 0.02 ^a	1.11 ± 0.03 ^a	1.53 ± 0.07 ^b	2.19 ± 0.08 ^c	3.09 ± 0.17 ^d	0.001	0.001	0.001	0.001	0.001	0.165	0.438
Soleus (mg/mm)	F	0.70 ± 0.04 ^{a,*}	0.87 ± 0.03 ^{a,b,***}	0.89 ± 0.04 ^{a,b,*}	1.07 ± 0.05 ^{b,***}	1.53 ± 0.07 ^{c,***}	2.42 ± 0.07 ^{d,***}							
	M	0.38 ± 0.02 ^a	0.42 ± 0.03 ^a	0.41 ± 0.02 ^a	0.49 ± 0.03 ^{a,b}	0.59 ± 0.03 ^{b,c}	0.68 ± 0.06 ^c	0.001	0.001	0.001	0.563	0.673	0.809	0.445
Heart (mg)	F	0.27 ± 0.02 ^{a,***}	0.35 ± 0.02 ^a	0.33 ± 0.02 ^{a,*}	0.36 ± 0.02 ^{a,b,***}	0.46 ± 0.03 ^{b,c,***}	0.55 ± 0.03 ^{c,***}							
	M	135.5 ± 7.1	140.8 ± 7.5	145.2 ± 5.6	147.1 ± 5.0	138.6 ± 7.5	140.8 ± 6.1	0.392	0.561	0.001	0.781	0.721	0.761	0.900
Heart (mg/g)	F	105.7 ± 2.9 [*]	111.9 ± 5.2 [*]	111.9 ± 6.3 ^{**}	112.9 ± 4.7 ^{**}	113.9 ± 4.7	109.5 ± 8.0 [*]							
	M	4.53 ± 0.16 ^a	4.37 ± 0.21 ^a	4.59 ± 0.12 ^a	4.02 ± 0.09 ^a	3.65 ± 0.09 ^b	3.01 ± 0.09 ^b	0.001	0.001	0.703	0.947	0.158	0.193	0.536
Tibia (mm)	F	4.67 ± 0.19 ^a	4.09 ± 0.14 ^a	4.55 ± 0.26 ^a	4.02 ± 0.12 ^a	3.74 ± 0.11 ^b	2.81 ± 0.09 ^c							
	M	18.5 ± 0.2	18.1 ± 0.1	18.3 ± 0.3	18.4 ± 0.2	18.3 ± 0.2	18.1 ± 0.2	0.469	0.881	0.222	0.937	0.221	0.099	0.549
NA length (mm)	F	18.0 ± 0.2	18.3 ± 0.1	18.0 ± 0.2	18.4 ± 0.2	18.1 ± 0.1	17.9 ± 0.2							
	M	99.3 ± 1.4	98.4 ± 0.8	98.3 ± 0.8	100.7 ± 0.7	100.5 ± 1.1	100.2 ± 1.4	0.017	0.102	0.001	0.558	0.297	0.309	0.452
	F	91.9 ± 1.0 ^{***}	94.8 ± 1.2 [*]	92.8 ± 1.2 ^{***}	95.2 ± 1.3 ^{**}	96.4 ± 1.0 [*]	96.4 ± 1.5 [*]							

Unlike letters denote significant differences among genotype within sex for a given muscle (at least $P < 0.05$). Daggers and asterisks indicate significant differences between sex within genotype for a given muscle ($^{\dagger}P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

B. fem, B. femoris; EDL, extensor digitorum longus; Gast, gastrocnemius; I, IGf1; M, MSTN; S, sex; T. ant, T. anterior.

Figure 6

Myofibre phenotype of WT and transgenic mice at 12 weeks (study 2). (A) A representative image of an SDS-PAGE gel showing the separation of myosin heavy chains (MyHC). The control sample (L) was from a 50:50 mixture of *soleus* and *EDL* muscles from mice for use as a reference. (B) Percent of MyHC 2A/X, (C) MyHC 2B and (D) MyHC 1 from the optical densities (OD) in *quadriceps* muscles of male and female *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{+/-} (*M*^{+/-}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{+/-}:*lgf1*⁺ (*M*^{+/-}:I⁺), *Mstn*^{+/-}:*lgf1*⁺ (*M*^{+/-}:I⁺) and *Mstn*^{-/-}:*lgf1*⁺ (*M*^{-/-}:I⁺) mice (*n* = 8 per genotype and sex). The mean concentration for each group is represented by the horizontal bar. Unlike letters denote significant differences between genotypes (at least *P* < 0.05). Asterisks indicate significance between sex within genotype (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

Discussion

We show here that increased expression of *Igf1Ea* and *Igf1r* mRNA in *gastrocnemius* coincides with the most rapid rate

Figure 7

Concentrations of IGF1 protein in plasma (A) and arbitrary concentrations of *Igf1* receptor mRNA (B) in *quadriceps* muscles of male and female *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{+/-} (*M*^{+/-}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{+/-}:*lgf1*⁺ (*M*^{+/-}:I⁺), *Mstn*^{+/-}:*lgf1*⁺ (*M*^{+/-}:I⁺) and *Mstn*^{-/-}:*lgf1*⁺ (*M*^{-/-}:I⁺) mice (*n* = 8 per genotype and sex, sampled at 12 weeks – study 2). The mean concentration for each group is represented by the horizontal bar. Asterisks indicate significance between sex within genotype (***P* < 0.01, ****P* < 0.001). Unlike letters denote significant differences among genotypes (*P* at least < 0.05).

of body growth and accretion of muscle mass in both male and female WT mice (study 1). The decline in expression of both genes after 6 weeks of age suggests that minimal transcription of these genes is required to maintain adult body and muscle mass, and/or that other factors are responsible for maintaining the mass of adult tissues. The pattern of *Igf1Ea* and *Igf1r* mRNA in *gastrocnemius* muscles is broadly consistent with that of concentrations of IGF1 protein in blood, although the amplitude of the latter is greatly reduced.

While concentrations of IGF1 protein in blood were not significantly different between sexes, or across time, the early peak is broadly consistent with the noted peripubertal increase in rodents between 4 and 6 weeks of age (Handelsman *et al.* 1987, Mathews *et al.* 1988, Hu *et al.* 1993). Puberty in mice occurs around 4.5 weeks (Safrański *et al.* 1993). The lack of differences between the sexes is consistent with local IGF1 playing the dominant role in regulating post-natal growth (Sjogren *et al.* 1999, Yakar *et al.* 1999, Klover & Hennighausen 2007).

Figure 8

Representative Western blots of total (t) AKT, pAKT^{S473} and GAPDH in quadriceps muscles of male (M) and female (F) *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{-/-}:*Igf1*⁺ (*M*^{-/-}:*I*⁺), *Mstn*^{+/+}:*Igf1*⁺ (*M*^{+/+}:*I*⁺) and *Mstn*^{-/-}:*Igf1*⁺ (*M*^{-/-}:*I*⁺) mice (*n* = 8 per genotype and sex, sampled at 12 weeks - study 2).

The timing of the increased expression of *Igf1Ea* mRNA in skeletal muscle, and the increase in concentrations in blood observed here and noted by others above, occurs after the mitotic phase of myogenesis, which, in mice, is completed within 3 weeks of post-natal life (Beilharz *et al.* 1992). This is consistent with IGF1 acting to increase hypertrophy of muscle fibres rather than regulating hyperplasia. In contrast, reduced abundance, or absence of myostatin increases hyperplasia of myofibres (Hennebry *et al.* 2017).

Our finding here of a lower expression of *Igf1* mRNA and delayed peak in *Igf1r* mRNA in skeletal muscles of juvenile female mice indicated that greater growth might occur if *Igf1* expression was increased during post-natal growth. Moreover, we had anticipated that growth of body and muscle mass may be greater in females than in males and that the difference in sexual dimorphism of body size would be reduced, or abolished in the current study. However, while transgenic mice had greater rates of body growth and eventual body and muscle mass, the difference in sexually dimorphic size persisted. Indeed, the mass of female *Mstn*^{-/-}:*Igf1*⁺ mice was ~30% greater, and the mass of the quadriceps was 2.5-fold greater, than male *Mstn*^{+/+} mice. However, within genotype, females remained ~25% smaller than males with a similar reduction in muscle mass than that of males.

The expression of the *Igf1* transgene, driven by the MLC promoter, is activated after 10 days post-partum (Musaro *et al.* 2001). Therefore, the increased production and availability of transgenic IGF1 early in post-natal skeletal muscle likely exacerbates the increase in mass of growing muscles. In confirmation, we found that

Figure 9

Optical densities of (A) total (t) AKT, (B) phosphorylated (p) pAKT^{S473} and (C) the ratio of p:tAKT in quadriceps muscles of male and female *Mstn*^{+/+} (*M*^{+/+}), *Mstn*^{-/-} (*M*^{-/-}), *Mstn*^{-/-}:*Igf1*⁺ (*M*^{-/-}:*I*⁺), *Mstn*^{+/+}:*Igf1*⁺ (*M*^{+/+}:*I*⁺) and *Mstn*^{-/-}:*Igf1*⁺ (*M*^{-/-}:*I*⁺) mice as determined by Western blotting (*n* = 8 per genotype and sex, sampled at 12 weeks - study 2). The mean concentration for each group is represented by the horizontal bar. The asterisk indicates a significance between sex for the *M*^{+/+} genotype (**P* < 0.05).

growth rates, body and muscle mass of male mice were greatly increased when myostatin was absent and IGF1 was present in excess (Hennebry *et al.* 2017). However, in female mice, a persistent partial refractoriness to IGF1 signalling via AKT may underlie the failure for the full potential growth to become manifest (see below).

Expression of transgenic *Igf1Ea* mRNA was at least 2-fold greater in quadriceps muscles of males than of females, which may be attributed to the greater abundance of type 2B MyHC myofibres because the MLC/*Igf1* transgene is

expressed in a hierarchical fashion in the order 2B, 2X and 2A (Shavlakadze *et al.* 2005). However, there was on average 1.4-fold more IGF1 protein in *quadriceps* muscles of females. This observation may point to a greater sensitivity of a negative feedback loop to reduce transcription of *Igf1Ea* mRNA in males, or that there is partial refractoriness to IGF1 in female skeletal muscles (and other tissues). The modest growth of *soleus* muscles is consistent with reduced expression of *Igf1Ea* mRNA and action in type 2A myofibres. *Soleus* muscles are roughly 40% type I and 60% type 2A (Augusto *et al.* 2004).

Concentrations of IGF1 were increased in blood of male but not female mice carrying the *Igf1* transgene. This result departs from our previous report (Hennebry *et al.* 2017), wherein we were unable to resolve differences in concentrations of IGF1 in blood between the six male genotypes. However, we did observe numerical increases in concentrations of IGF1 in blood of *Mstn^{+/+}:Igf1⁺* and *Mstn^{-/-}:Igf1⁺* mice. The same assay was used again but with different dilutions to measure the higher concentrations of IGF1 in the transgenic mice. This time, the increased concentrations in IGF1 transgenic males were apparent. It should be noted that the assay does not discriminate between transgenic and endogenous forms of IGF1 but at 12 weeks of age, we speculate that the increased concentrations are due to the presence of the transgene and not due to an increase in endogenous secretion. The increased circulating concentrations of IGF1 in male mice transgenic for *Igf1* may contribute to the greater growth in these mice. As noted in the Introduction, while local expression of *Igf1* has the predominant effect on growth, circulating concentrations can contribute up to 30% of post-natal growth and adult body size (Stratikopoulos *et al.* 2008).

Expression of the *Igf1r* mRNA had no clear pattern and was not consistently affected by the absence of myostatin or the presence of the IGF1 transgene. However, expression was increased in female *quadriceps* muscles of *Mstn^{+/+}*, *Mstn^{+/+}:Igf1⁺*, *Mstn^{-/-}* and reduced in muscles of both sexes when myostatin was absent and in the presence of the *Igf1* transgene (*Mstn^{-/-}:Igf1⁺*). The pattern in males is broadly similar to our previous observation wherein *Igf1r* mRNA was reduced in male *quadriceps* muscles of *Igf1* transgenic mice (Hennebry *et al.* 2017). Given that the expression of *Igf1r* mRNA declines after 6 weeks of age and is low at 12 weeks of age (study 1), when these tissues were collected in study 2, we speculate that the mRNA reflects basal expression in adults rather than expression at peak growth.

The decline in the mass of the gonadal fat pad with the increased lean mass is consistent with a combined

effect of the loss of functional myostatin (Hamrick *et al.* 2006) and an increase in IGF1 (Fiorotto *et al.* 2003) in promoting a lean phenotype. Myostatin directs the fate of precursor cells towards a lipogenic lineage (Artaza *et al.* 2005), while IGF1 promotes a myogenic fate via regulating Rho GTPase (Sordella *et al.* 2003).

Tibia length remained the same within sex and genotype, which is consistent with previous reports (Shimizu & Awata 1984, Hennebry *et al.* 2017). Other than the humerus, leg bones do not exhibit sexually dimorphic growth (Shimizu & Awata 1984). However, the vertebral bones grow at a faster rate in male, than in female mice (Shimizu & Awata 1984), which is consistent with the longer nose-to-anus length observed in males in the current study and in others (Klover & Hennighausen 2007).

Normalising the mass of the heart to body mass, or to tibia length raised to the power of 3, has now been shown to be a better method than to tibia length alone (Hagdorn *et al.* 2019) and we used this method here. However, the mass of the heart in *Mstn^{-/-}* mice has been reported to be unchanged from WT mice up to 100 days of age (Rodgers *et al.* 2009). Our data accord with the lack of differences in uncorrected heart mass among genotypes at 12 weeks of age but show that there is a sexually dimorphic difference wherein females have smaller hearts than males. After correcting to body mass though, the differences between sexes disappear. Therefore, the decreased mass of the hearts corrected to body mass in *Mstn^{-/-}* and *Mstn^{-/-}:Igf1⁺* are an artefact of greater body mass alone.

We observed that the absence of myostatin together with the presence of transgenic-derived IGF1 altered the myofibre phenotype. The composition of MyHC in myofibres determines the fast- and slow-twitch characteristics of skeletal muscle. Our current findings accord with those of others who showed that *Mstn^{-/-}* mice have more myofibres expressing type 2 MyHC in the *soleus* (type 2A) and *EDL* muscles (types 2B and 2X) (Girgenrath *et al.* 2005, Amthor *et al.* 2007) and that IGF1 directly upregulates the type 2B MyHC promoter (Shanely *et al.* 2009). Our data also accord with the use of the MLC promoter, which is most active in muscles expressing type 2B MyHC and least in the type 1 MyHC expressing myofibres (Donoghue *et al.* 1991, Musaro *et al.* 2001).

The increased abundance of total AKT in conjunction with decreased copy number of *Mstn* in *quadriceps* muscles and the increase in phosphorylated AKT in association with mice transgenic for *Igf1* is consistent with our previous report (Hennebry *et al.* 2017). We now report that there is a greater abundance of total AKT in *quadriceps* in

females as compared with males, while pAKT^{S473} remains largely unchanged between sexes. Therefore, the ratio of p-t AKT is greater in *quadriceps* of males than females, which is consistent with sexually dimorphic growth of skeletal muscles. The increased expression of *Igf1r* mRNA in muscles of female mice transgenic for *Igf1* is consistent with reduced negative feedback regulation from the decreased activation of AKT, which likely acts on mTORC1 to inhibit and degrade IRS1 (Rozenfurt *et al.* 2014).

It was recently reported that IGF2 may contribute to the hypermuscular muscle development in *Mstn*^{-/-} mice because concentrations of *Igf2* mRNA and protein were higher in muscles of *Mstn*^{-/-} compared with WT mice from 3 to 10 weeks of age (Clark *et al.* 2015). We did not measure concentrations of *Igf2* mRNA or protein in the current study, but presume that they would have been similarly increased in skeletal muscles of *Mstn*^{-/-} mice used here. If so, the increased concentrations of IGF2 protein may have contributed to the additional growth of body and muscle mass in *Mstn*^{-/-} mice. However, we believe that it is unlikely that IGF2 plays an important role in regulating sexually dimorphic growth given that sexual dimorphism persisted in this study and that by Clark *et al.* (2015).

In conclusion, we report that excess IGF1, in conjunction with the absence of myostatin, greatly increased the body and muscle mass of both sexes and promoted a leaner phenotype with faster twitch myofibre characteristics. However, neither the absence of myostatin nor excess IGF1 nor the combination of the two overcame, or reduced sexually dimorphic growth. Instead, sexual dimorphism persisted with males remaining consistently larger than females – a phenomenon that is consistent with greater IGF1-induced activation of AKT in skeletal muscles of males.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C D M, F J, H K S and R P interpreted the data and wrote the manuscript. J O, S F, K W, M T and collected and analysed the data. C D M, K G M and T W conducted the animal experiments.

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