Regulation of sexually dimorphic growth of murine skeletal muscle by Stat5a and Stat5b

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Introduction

- Growth hormone (GH) regulates insulin-like growth factor one (IGF-1) predominantly through the intracellular signallers Stat5a and Stat5b.[1]
- Inactivating mutations of Stat5b in humans results in severe growth retardation and low circulating concentrations of IGF-1 in both sexes.[2]
- Deletion of Stat5b in mice results in loss of sexually dimorphic growth with a reduction of growth and circulating concentrations of IGF-1 in males only.[3,4]
- The reasons for the discrepancy in the role of Stat5b between humans and mice is not known.
- No study has observed Stat5b$^{-/-}$ mice beyond 12 weeks of age or investigated any subsequent changes in the mass of skeletal muscles or expression of IGF-1.

Aims

- To determine the regulation of the sexually dimorphic growth of skeletal muscles in mice by Stat5b.
- To determine the regulation of expression of IGF-1 mRNA in skeletal muscles of mice by Stat5a and Stat5b.

Study design

- Blood and hindlimb muscles of male and female Stat5b$^{-/-}$ mice and wild-type littermates were collected at 6, 12 and 24 weeks of age.
- n = 16 per age and sex.
- Concentrations of IGF-1 in plasma and skeletal muscle were determined by ELISA & muscle mass was normalised to bone length.
- C_2C_{12} myoblast cell lines were treated with viral Stat5b and/or Stat5a siRNA or a scrambled vector (control), then differentiated and treated with GH 100 ng/mL for 24 hours (n=6). RNA was harvested for qPCR.
- The siRNA treated cell lines were also treated with GH 100 ng/mL for 96 hours and differentiation was assessed by immunocytochemistry.

Table 1: Mean length (+/- S.E.M.) of the tibia (mm) and concentrations of IGF-1 in plasma and skeletal muscle relative to male WT mice at 6 weeks of age

Age	Sex	Genotype	Tibia length	P*	Plasma IGF-1	P*	Muscle IGF-1	P*
6 weeks	M	WT	16.8 +/- 0.2	a	100 +/- 8.4	a	100 +/- 7.3	a
	M	Stat5b$^{-/-}$	15.8 +/- 0.2	b	56.3 +/- 4.0	b	43.8 +/- 2.4	b
	F	WT	16.4 +/- 0.2	a	107.6 +/- 6.3	a	79.9 +/- 7.8	c
	F	Stat5b$^{-/-}$	15.9 +/- 0.1	b	79.9 +/- 3.9	c	64.2 +/- 5.5	d
12 weeks	M	WT	18.3 +/- 0.2	a	93.2 +/- 4.7	a	106.0 +/- 6.0	a
	M	Stat5b$^{-/-}$	16.6 +/- 0.2	b	57.2 +/- 4.6	b	48.3 +/- 4.6	b
	F	WT	17.7 +/- 0.2	a	91.2 +/- 5.4	ac	67.6 +/- 6.6	c
	F	Stat5b$^{-/-}$	16.7 +/- 0.2	b	80.8 +/- 2.1	c	63.6 +/- 5.1	c
24 weeks	M	WT	18.1 +/- 0.1	a	101.8 +/- 5.5	a	79.6 +/- 5.9	a
	M	Stat5b$^{-/-}$	17.2 +/- 0.1	b	77.6 +/- 4.3	b	47.9 +/- 2.2	b
	F	WT	18.4 +/- 0.2	a	107.8 +/- 5.6	a	64.1 +/- 3.3	c
	F	Stat5b$^{-/-}$	17.4 +/- 0.1	b	99.9 +/- 7.5	a	58.3 +/- 4.6	bc

* Different letters denote significance ($P < 0.05$) between each group at each age only

References

1) Herrington et al. Oncogene 2000; 19(21): 2585-97
2) Hwa et al. BPRCEM 2001; 25(1): 61-75.
3) Teglund et al. Cell 1998; 93(5): 841-850.
4) Udy et al. PNSA 1997; 94(14): 7239-7244.

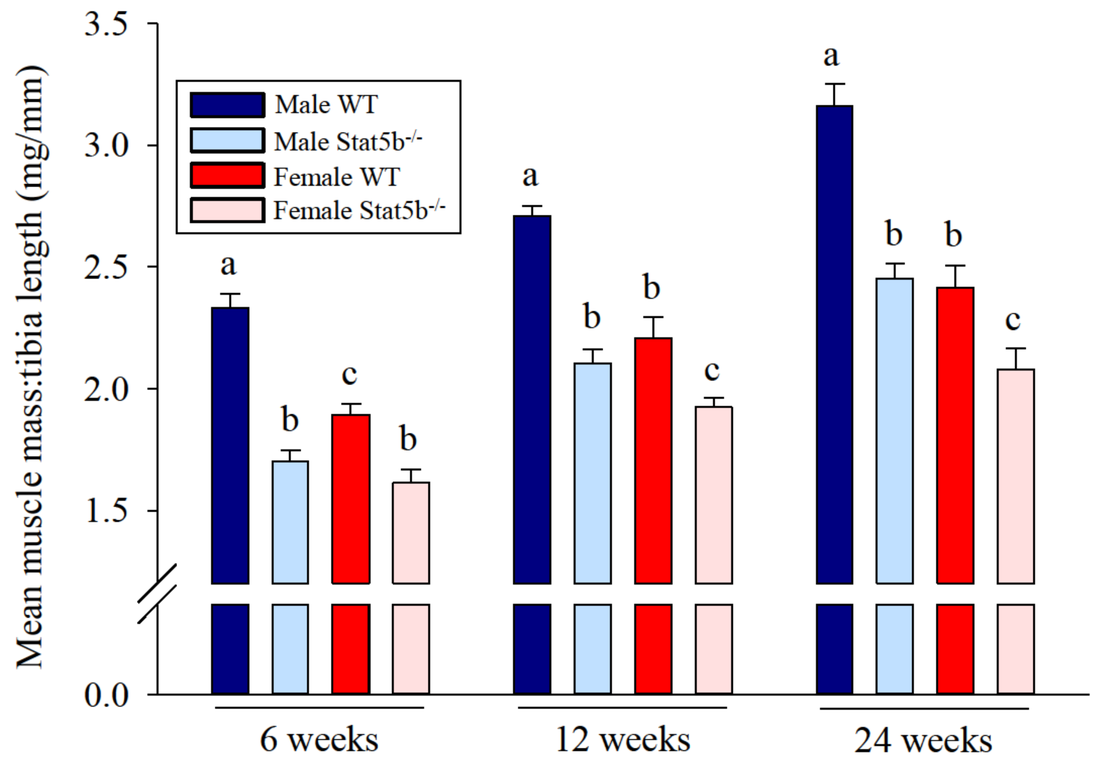

Figure 1: Normalised mass of tibialis anterior muscles

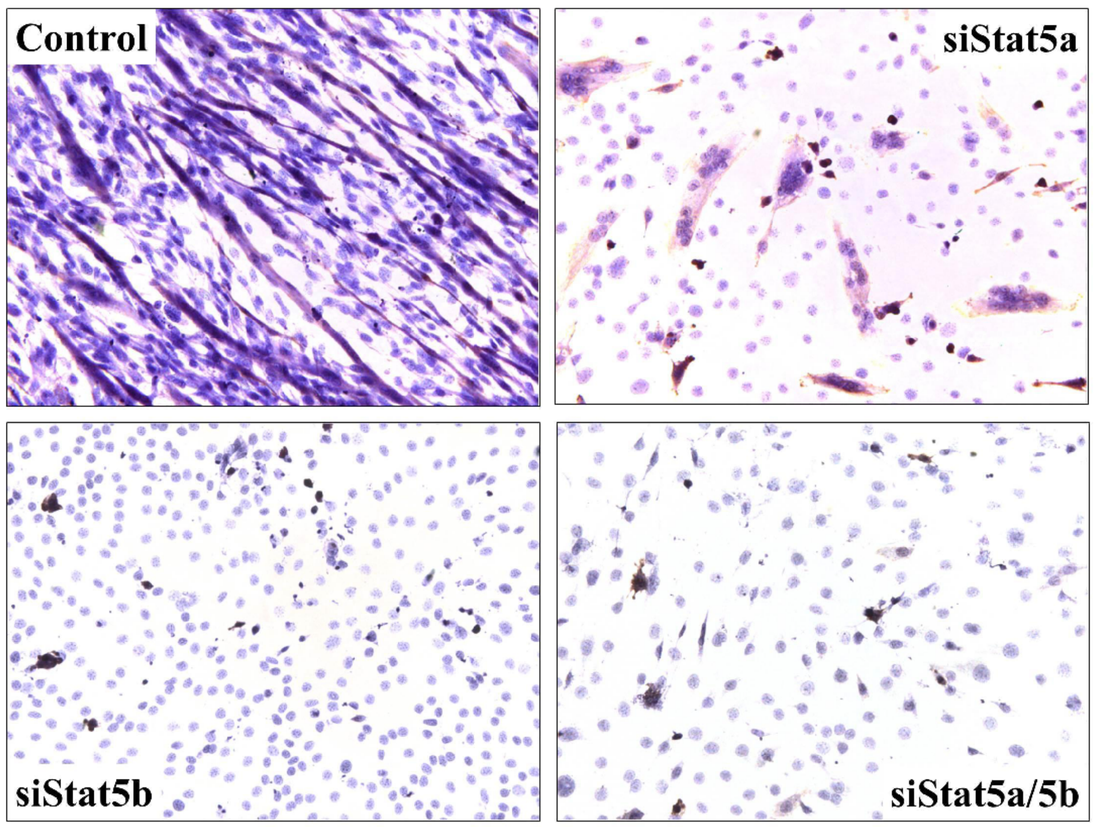

Figure 2: 10 x magnification of siRNA treated myoblasts at 96 hours differentiation

Results

- Nasoanal length, tibia length and the normalised mass of hindlimb muscles were reduced to a greater extent in male (23%) than in female (14%) Stat5b$^{-/-}$ mice at all ages ($P < 0.001$; Figure 1 and Table 1).
- Concentrations of IGF-1 in plasma and skeletal muscle were reduced in male Stat5b$^{-/-}$ mice at all ages and in female Stat5b$^{-/-}$ mice at only 6 weeks of age ($P < 0.01$ versus wild-type littermates of the same sex).
- Knockdown of Stat5a alone reduced the differentiation of myotubes (36%) to a lesser extent than knockdown of Stat5b or both Stat5a and Stat5b (80%; $P < 0.001$; Figure 2).
- Knockdown of either Stat5a or Stat5b prevented the GH-induced increase in concentrations of IGF-1 mRNA and myotube hypertrophy

Conclusions

- **Stat5a and Stat5b are both required for signaling of GH in skeletal muscles of mice.**
- **Similar to humans, loss of function of Stat5b in mice is associated with retarded growth and reduced circulating concentrations of IGF-1 in both males and females.**
- **Sexually dimorphic growth of skeletal muscles was reduced, but persisted in Stat5b$^{-/-}$ mice.**

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