Regulation of murine skeletal muscle mass by testosterone and 17β-estradiol

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Introduction

- The predominant positive and negative regulators of the mass of skeletal muscles are insulin-like growth factor one (IGF-1) and myostatin (Mstn), respectively.
- The regulation of the mass of skeletal muscles and activity of IGF-1 and Mstn by the gonadal steroids testosterone (T) and 17β-estradiol (E_2) remains controversial.

Aims

- To determine the regulation of the mass of skeletal muscles by T and E_2 in male and female mice.
- To determine the regulation of the activity of IGF-1 and Mstn by T and E_2 in male and female mice.

Study design

- Male and female mice (C57BL/6 strain) underwent bilateral gonadectomy (Gdx) or sham surgery at 4 weeks of age with insertion of subcutaneous silastic implants containing T, E_2 or cholesterol (placebo) (n = 8 per treatment and sex).
- Blood and hindlimb muscles were collected at 13 weeks of age.
- Muscle mass was normalised to acting bone length.
- Concentrations of IGF-1 in plasma and skeletal muscle were determined by ELISA.
- C_2C_{12} myoblasts under differentiating conditions were treated for 24 hours with T (30 nM) or E_2 (10 nM) and RNA and protein were harvested for quantitative PCR and Western blotting, respectively.
- Myoblasts were also treated for 96 hours and differentiation was assessed by immunocytochemistry.

Results

- Sexually dimorphic growth of hindlimb muscles was abolished post-gonadectomy (Figure 1).
- Replacement of T to male mice prevented the gonadectomy-induced reduction in normalised mass of hindlimb muscles and concentrations of IGF-1 protein and the gonadectomy-induced increase in the abundance of mature Mstn protein (Table 1).
- Administration of E_2 to male mice attenuated the gonadectomy-induced reduction in the normalised mass of hindlimb muscles, but did not alter concentrations of Mstn or IGF-1 in skeletal muscle or plasma.
- Bilateral gonadectomy +/- replacement of E_2 in female mice did not alter the abundance of mature Mstn protein or the normalised mass of hindlimb muscles, despite decreasing concentrations of IGF-1 in plasma and skeletal muscle.
- Administration of T to female mice increased the normalised mass of hindlimb muscles and concentrations of IGF-1, whilst decreasing the abundance of mature Mstn protein in skeletal muscle.
- Administration of T increased the hypertrophy of C_2C_{12} myotubes (149%) to a greater extent than administration of E_2 (61%; Fig. 2).
- Expression of IGF-1 mRNA in C_2C_{12} myotubes was increased by administration of T, but not by administration of E_2 (Figure 3).

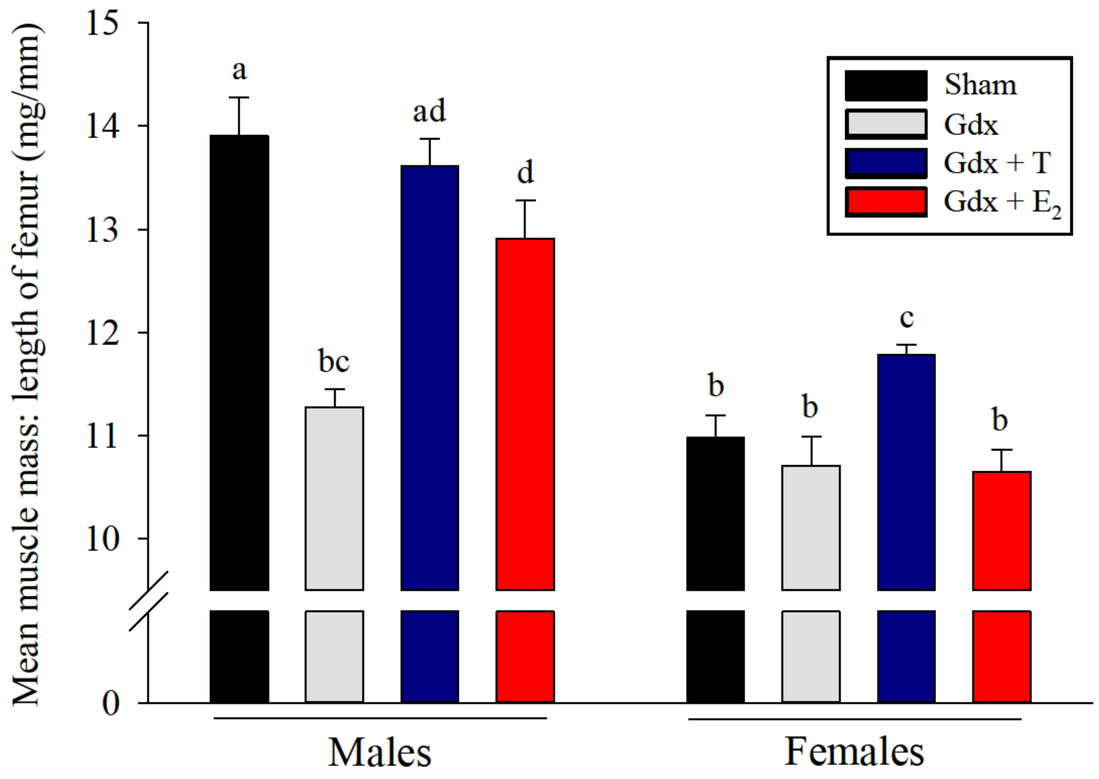

Figure 1: Normalised mass of quadriceps muscles

Table 1: Mean concentrations (+/- S.E.M.) of IGF-1 in plasma and IGF-1 and mature Mstn protein in skeletal muscle relative to the male sham group

Sex	Group	Plasma IGF-1	P*	Muscle IGF-1	P *	Mstn	P*
M	Sham	100 +/- 8.2	ab	100 +/- 7.2	a	100 +/- 6.0	a
	Gdx	95.8 +/- 6.0	abc	67.9 +/- 4.3	b	157.9 +/- 6.8	b
	Gdx + T	91.5 +/- 6.2	ab	85.3 +/- 4.4	c	67.2 +/- 6.4	c
	Gdx + E_2	109.8 +/- 7.9	a	67.8 +/- 3.4	bd	145.4 +/- 13.8	b
F	Sham	101.6 +/- 4.1	a	70.6 +/- 7.2	b	175.6 +/- 14.6	b
	Gdx	85.0 +/- 5.1	bc	54.0 +/- 2.9	e	170.8 +/- 16.0	b
	Gdx + T	81.0 +/- 4.0	c	67.0 +/- 1.8	bd	94.8 +/- 9.1	a
	Gdx + E_2	87.6 +/- 5.6	bc	55.8 +/- 3.3	de	153.0 +/- 9.9	b

* Different letters denote significance ($P < 0.05$) between each group in both male & female mice

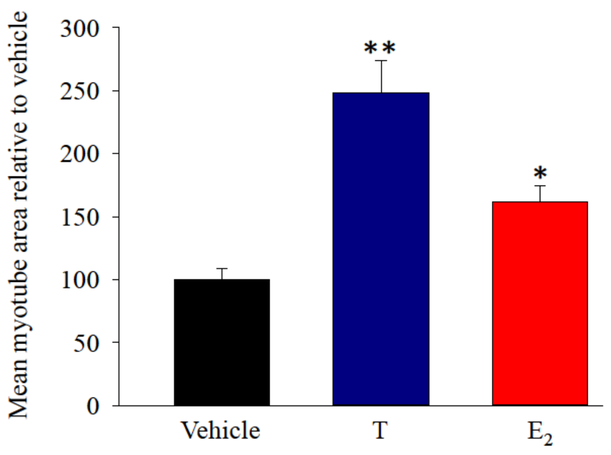

Figure 2: Mean myotube area

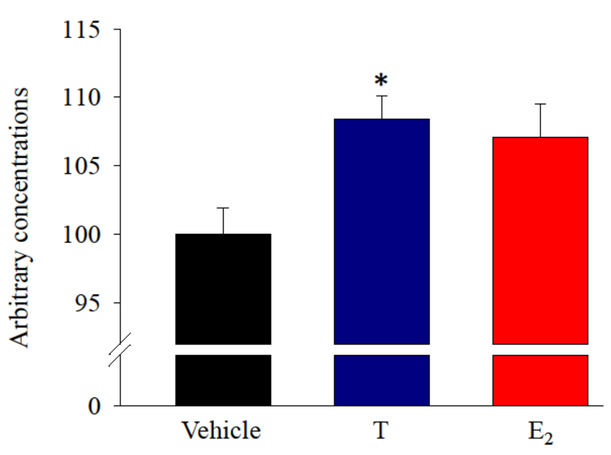

Figure 3: Myotube IGF-1 mRNA

Conclusions

- **The anabolic action of T on skeletal muscles in mice is at least in part, due to modulation of activity of IGF-1 and Mstn.**
- **The E_2-induced increase in mass of skeletal muscles occurred in male mice only and appeared to be independent of IGF-1 or Mstn.**
- **Gonadal steroids regulate the sexually dimorphic growth of skeletal muscles in mice.**

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