Thymosin beta 4 enhances myogenesis *in vitro* but does not confer regenerative advantage on skeletal muscle *in vivo* following myotoxic injury

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Thymosin β4 (Tβ4) is a small, ubiquitously expressed peptide that possesses a broad range of biological activities, and has been shown to reduce inflammation, enhance angiogenesis, and improve regeneration in a number of tissues including dermis, cornea, cardiac muscle and brain. Recent studies have shown that Tβ4 is upregulated in skeletal muscle after injury, can enhance myoblast migration *in vitro*, and is upregulated during myoblast differentiation (Tokura *et al.*, 2011), suggesting a role for Tβ4 in post-injury muscle regeneration. In addition to the full-length peptide there are three naturally occurring biologically active peptide fragments of Tβ4, each with their own characteristic biological activities (Sosne *et al.*, 2010).

The aim of this study was to examine the effect of Tβ4 and its fragment peptides on muscle regeneration following injury. We hypothesized that Tβ4 would enhance myoblast proliferation and differentiation *in vitro*, and also enhance skeletal muscle regeneration following myotoxic injury *in vivo*. *In vitro* experiments were performed with C2C12 myoblasts, and we assessed the influence of full-length Tβ4 and its fragment peptides on myoblast proliferation, chemotaxis, and differentiation. All *in vivo* experiments were approved by the Animal Ethics Committee of La Trobe University and conducted in accordance with the codes of practice stipulated by the National Health and Medical Research Council (Australia). C57BL/10 mice (12 wks old) were anaesthetized (4% isoflurane in O_2, 2L/min *via* inhalation), and the *tibialis anterior* (TA) muscle of the right hindlimb was injected with Cardiotoxin (CTX) to cause complete muscle fibre degeneration. Mice were allowed to recover for 7, 14 or 21 days, during which they received twice weekly treatments with either full-length Tβ4 (6mg/kg, *i.p*) or saline control. After the recovery period mice were anaesthetized (60 mg/kg, sodium pentobarbital, *i.p.*) and TA muscle function was assessed *in situ* as reported previously (Gehrig *et al.*, 2010). At the conclusion of the experiment mice were killed by cardiac excision while still anaesthetized deeply.

We found that full-length Tβ4 and two of its active peptides (Tβ4[1-4] and Tβ4[1-15]) enhanced myoblast proliferation, chemotaxis and differentiation *in vitro*, however when the full-length Tβ4 peptide was administered to mice following myotoxic injury it failed to significantly alter tetanic force production or twitch characteristics in regenerating muscles *in vivo*. These results suggest that although Tβ4 enhances myogenesis *in vitro*, increases in endogenous Tβ4 expression during regeneration *in vivo* may produce sufficient levels of the peptide such that exogenous administration of further Tβ4 may be redundant.

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Sosne G, Qiu P, Goldstein AL & Wheater M. (2010) *FASEB Journal* **24**, 2144-2151.
Tokura Y, Nakayam Y, Fukada S, Nara N, Yamamoto H, Matsuda R & Hara T. (2011) *Journal of Biochemisty* **149**, 43-48.