Effect of mechanical stretching on Akt signalling and protein synthesis in myotubes

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Introduction. Recently, studies using pharmacological and genetic modifications in rodents and cells have found the protein kinase Akt (protein kinase B) to be a major regulator of muscle mass. When active, Akt increases protein synthesis *via* signalling through the mammalian target of rapamycin (mTOR) and glycogen synthase kinase-3β (GSK-3β), and inhibits protein degradation *via* forkhead box, sub-group O (FOXO) family of transcription factors (Glass, 2005). *In vitro* mechanical stretch mimics the effects of resistance exercise (Powell *et al.*, 2002) and has been used to investigate the influence of mechanical load on myotube protein synthesis (Vandenburgh *et al.*, 1989) and more recently, Akt activation (Hornberger *et al.*, 2005). However, whether mechanical stretch causes an increase in protein synthesis *via* Akt signalling has not been determined. This study aimed to determine whether the increase in Akt signalling seen in response to ten minutes of stretch (Hornberger *et al.*, 2005) results in an increase in protein synthesis, and whether the increase in protein synthesis seen in response to 48 hours of stretch (Vandenburgh *et al.*, 1989) was associated with an increased activation of the Akt signalling pathways.

Methods. C2C12 myoblast cultures were grown and differentiated into myotubes on Bioflex 6-well flexible bottom culture plates. Myotubes were mechanically stretched using the Flexcell strain unit (FX-4000) for either 10 min at 15% stretch (Hornberger *et al.*, 2005) or 48 h at 8% stretch (Vandenburgh *et al.*, 1989). Phosphorylated and total proteins for Akt, p70S6k, GSK-3β, and FOXO were measured using western blotting techniques. Atrogin-1 and MuRF1 mRNA levels were measured using real time-PCR. Protein synthesis rates were measured by the incorporation of [^{3}H]-tyrosine into the myotubes.

Results. Ten minutes of stretch caused an increase in phosphorylated Akt, p70S6k and GSK-3β and a decrease in Atrogin-1 and MuRF1 mRNA when measured immediately post-stretch. Protein synthesis rates, measured during the first 2 h post stretch, did not change. 48 h of stretch resulted in a decrease in phosphorylated Akt, p70S6k and GSK-3β as well as Atrogin-1 when measured immediately post-stretch. This was associated with a 12% increase in synthesis rates, measured during the last 2 h of stretch.

Conclusion. Short duration high-intensity mechanical stretching increases the phosphorylation of members of the Akt signalling pathway, but does not result in increases in protein synthesis within 2 h post stimulation. A longer time period of protein synthesis may be required. In contrast, longer duration low-intensity mechanical stretching results in a decrease in the phosphorylation of members of the Akt signalling pathway, but does result in increased protein synthesis during the last two hours of stimulation. The reduction in Akt signalling may be caused by a feed-back mechanism aimed at minimising excessive protein synthesis levels.

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