Regulation of atrogin-1 and protein degradation following incubation with dexamethasone and TNFα in mouse C2C12 and primary human myotubes

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Introduction: Atrogin-1 is a muscle specific E3-ligase involved in muscle wasting (Bodine *et al.,* 2001). Increased levels of atrogin-1 mRNA has been observed in numerous *in vitro* and *in vivo* rodents models of muscle atrophy (Glass, 2005). Human studies performed have shown that atrogin-1 is increased in human atrophy conditions, such as leg immobilization (Jones *et al.,* 2004), ALS (Leger *et al.,* 2006), COPD (Doucet *et al.,* 2007) and quadriplegic myopathy (Di Giovanni *et al.,* 2004). In mice, but not humans, fasting increases atrogin-1 (Sandri *et al.,* 2004; Larsen *et al.,* 2006), suggesting that species differences may exists with respect to its regulation. The aim of the present study was to determine the regulation of atrogin-1 and protein degradation following treatment with dexamethasone (DEXA) and TNFα in mouse C2C12 and human primary myotubes.

Methods: Mouse C2C12 myotubes and primary human myotubes were treated with either TNF-α (20 ng/mL) or DEXA (10 μM) for 1, 4, 24 and 48-h. Atrogin-1 mRNA levels were measured using real time-PCR. Protein degradation was determined by measuring the release of [^{3}H]-tyrosine into the media.

Results: Atrogin-1 mRNA was significantly increased 2- and 4-fold in C2C12 myotubes after 24 and 48-h treatment with DEXA, respectively. In human myoblasts atrogin-1 was increased 2.2-fold only after 48-h of DEXA treatment. After treating C2C12 cells with TNF-α, atrogin-1 showed a transient change, increasing by 50% following 1-hr of treatment, decreasing to 50% below control levels following 4h of treatment then retuning to control levels after 24 and 48-h. In contrast, human myotubes treated with TNF-α showed a 3.1 fold increase in atrogin-1 after 48-h of treatment. In the human myotubes the increase in atrogin-1 mRNA levels following 48-h of both DEXA and TNF-α treatment resulted in significant increases in protein degradation by approximately 15%.

Conclusions: Treatment of both mouse C12C12 myotubes and primary human myotubes with DEXA results in increases in atrogin-1 mRNA; however human myotubes require a longer treatment period. Treatment with TNF-α demonstrated a more dramatic species dependent effect, with mouse C2C12 myotubes presenting a rapid increase, then decrease in atrogin-1 over 1-4 h of treatment, followed by a return to baseline lines at 48-h. In contrast, human myotubes had an increase in atrogin-1 mRNA 48- after treatment. In human myotubes the increases in atrogin-1 caused by both DEXA and TNF-α was associated with an increase in protein degradation. These observations highlight the need for caution when translating results obtained in rodent models to human conditions.

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