Ageing alters the inflammatory response in rat skeletal muscles after injury

C. van der Poel,[1] J.G. Ryall,[1] J.D. Schertzer,[1] L.E. Gosselin[2] and G.S. Lynch,[1] [1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY 14214, USA.

Some of the most serious consequences of ageing are its effects on skeletal muscle, characterized by wasting and weakness (Ryall *et al.* 2008). Although the mechanisms responsible for these changes have yet to be elucidated fully, an age-related impairment in regenerative capacity is a contributing factor (Carlson and Conboy, 2007). Successful muscle regeneration after injury requires a carefully regulated inflammatory response that removes damaged cells and initiates satellite cell activation (Charge & Rudnicki, 2004). However, the impact of ageing on the inflammatory/cytokine response of skeletal muscle after injury remains poorly understood. We investigated whether ageing affected the expression of inflammatory markers in injured/regenerating muscles and tested the hypothesis that the inflammatory process in injured muscles of old rats would be prolonged.

Male Fischer 344 rats aged 3 months (young, n = 20), 12 months (adult, n = 20) and 24 months (old, n = 20) were anaesthetised (100 mg/kg ketamine and 10 mg/kg xylazine i.p, 2 ml/kg). The extensor digitorum longus (EDL) muscles of the right hindlimb were injected with bupivacaine hydrochloride in the distal, proximal, and midbelly regions to ensure complete degeneration of all muscle fibres. The EDL muscle of the left hindlimb served as the non-injected uninjured control. EDL muscles were excised at either 12-, 24-, 36-, or 72-hours after bupivacaine injury. RT-PCR was used to determine mRNA expression levels of the inflammatory markers: TNFα, IFNγ, IL1β, IL18, IL6, and CD18. Gene expression was quantified using the cycle threshold (CT) method, before being normalised to the concentration of input cDNA. Relative gene expression was determined by comparisons with uninjured controls.

At 12 and 24 hours post-injury, all inflammatory cytokines were increased in regenerating muscles, but there was no significant difference between age groups. At 36 hours post-injury, there was a 2-3 fold increase in all cytokines examined in the muscles of old compared with young and adult rats. At 72 hours post-injury cytokine levels in regenerating muscles of young and adult rats were decreased but remained elevated (~10-fold higher) in regenerating muscles of old rats. These findings indicate that ageing is associated with an altered muscle inflammatory response after injury, which contributes to the age-associated decrease in muscle regenerative capacity.

Ryall JG, Schertzer JD, Lynch GS. (2008) *Biogerontology*, **9:** 213-28.
Carlson ME, Conboy IM. (2007) *Ageing Cell*, **6:** 371-82.
Charge SB & Rudnicki MA. (2004). *Cell*, **113:** 422-3.

Supported by research grant funding from the National Health and Medical Research Council (454561).