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Differential responses in muscle atrogene expression in a mouse model of critical illness

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Patients admitted to the Intensive Care Unit (ICU) often experience a loss of muscle mass and function, which increases their mortality and contributes to health deficits post-discharge. Treatments for critical illness induced myopathy remain elusive because of difficulties in conducting large, robust clinical trials in the ICU, difficulty in obtaining muscle biopsies and inter-patient variability. Hence, small animal models of critical illness can help address shortfalls in mechanistic understanding and developing effective treatments. The 'gold standard' *in vivo* model of critical illness is the Cecal Ligation and Puncture (CLP) model in mice, a poly-microbial model of peritoneal sepsis that recapitulates the inflammatory profile and loss of muscle mass experienced by human patients in the ICU (Seemann *et al.,* 2017; Zanders *et al.,* 2022). CLP mice undergo an initial pro-inflammatory phase followed by a compensatory anti-inflammatory phase. Combined with welfare support, such as antibiotics, analgesic, and fluid resuscitation, this model serves as a translatable model of critical illness induced muscle wasting.

Experiments were approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute and The University of Melbourne in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). 10–12-week-old C57BL/6 male mice underwent CLP surgery. Mice were anaesthetised using isoflurane (4% induction, 2% maintenance), and a laparotomy performed. The caecum was isolated, ligated, then punctured. The incision was closed, and during recovery the mice received warmed saline, antibiotics and analgesic three times daily until experimental endpoint. Mice were anaesthetised deeply using isoflurane (5%) and killed via cardiac puncture 48 hours after CLP surgery, with terminal collection of blood and hindlimb muscles for biochemical (mRNA) analyses of inflammation and atrogenes.

Forty-eight hours after induction of sepsis, differential responses to inflammation and atrogenes were evident in the hindlimb muscles (tibialis anterior, quadriceps, gastrocnemius, soleus and plantaris). All muscles had increased mRNA expression of atrophic markers *Atrogin-1* ($P<0.01$) and *Murf-1* ($P<0.05$) compared to muscles from non-surgical, age- and sex-matched control mice. In the soleus muscle, *Atrogin-1* mRNA was increased ~three-fold compared to control. This was considerably less than in other muscles, where *Atrogin-1* mRNA increased 25-, 23-, 20-, and 23-fold in the tibialis anterior, quadriceps, gastrocnemius, and plantaris muscles, respectively.

The CLP model in mice provides important insight into the mechanisms of sepsis-induced muscle wasting and weakness. The findings highlight the need to investigate muscles of different fibre composition, since some muscles may be better protected from inflammation and muscle wasting. This study also highlights the importance of investigating muscles of different fibre composition to determine therapeutic efficacy, since this may be influenced by muscle phenotype.

Seemann, S., Zohles, F., & Lupp, A. (2017). *Journal of Biomedical Science* **24**(1):60.
Zanders, L., Kny, M., Hahn, A., Schmidt, S., Wundersitz, S., Todiras, M., Lahmann, I., Bandyopadhyay, A., Wollersheim, T., Kaderali, L., Luft, F.C., Birchmeier, C., Weber-Carstens, S., & Fielitz J. (2022). *Journal of Cachexia, Sarcopenia and Muscle* **13**(1):713-27.