Inflammatory regulation by Selenoprotein S is not responsible for the loss of muscle performance

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Emerging interest surrounds the importance of selenoproteins in skeletal muscle growth, development and muscle performance (Moghadaszadeh *et al.*, 2013). Selenoprotein S (Seps1) is one of seven endoplasmic reticulum (ER) resident antioxidant selenoproteins and is highly expressed in skeletal muscle. Seps1 has been implicated in ER stress reduction, antioxidant defences and inflammation, where reduced Seps1 expression is thought to be associated with a heightened pro-inflammatory state. Our laboratory recently investigathed the role of Seps1 in dystrophic *mdx* mice, a model of increased muscle damage and inflammation. The genetic reduction of Seps1 by 50% amplified the inflammatory state of fast twitch EDL muscle and reduced myofibre size (Wright *et al.*, 2017). On a C57BL/6J background, the global genetic reduction or deletion of Seps1 was associated with reduced spontaneous physical activity and impaired isometric force output of isolated fast twitch EDL, but not slow twitch *soleus*, muscle *ex vivo*. However, the mechanistic understanding remains unknown. Thus, to further elucidate the role of Seps1 in skeletal muscle performance and inflammatory responses, adult Seps1 global knockout (GKO) knockout ($Seps1^{-/-}$), heterozygous ($Seps1^{+/-}$) mice and their wildtype ($Seps1^{+/+}$) littermates underwent a strenuous treadmill running protocol, as strenuous endurance exercise increases inflammatory, oxidative and ER stress, before *Tibialis anterior* (TA) muscle function was assessed *in situ* with an intact blood and nerve supply.

The animal studies were approved by the Animal Ethics Committee at La Trobe University, in accordance with NH&MRC guidelines. On 3 consecutive days, mice underwent a single bout of incremental exercise, starting at 5m/min and increasing in speed by 5 m/min, every 5 min to 25m/min or until voluntary cessation. Approximately 24 h following the third bout of exercise, mice were anesthetised *via* intraperitoneal injection of Sodium pentobarbital (60 μg/g), such that they were unresponsive to tactile stimuli. TA muscle function was assessed *in situ*, where the distal tendon was attached to a force transducer (1300A Whole Mouse Test System, Aurora Scientific), allowing assessment of isometric muscle force production, fatigueability following 4 min of intermittent stimulation (100 Hz, every 5 s) and force recovery. Following which, anaesthetized mice were humanely euthanized by cervical dislocation, and blood and tissues were collected for molecular and histological analysis.

The genetic reduction or deletion of Seps1 was associated with a reduction in the distance run and exercise completion rate in $Seps1^{-/-}$ and $Seps1^{+/-}$ mice compared with $Seps1^{+/+}$ littermates. $Seps1^{-/-}$ and $Seps1^{+/-}$ mice produced less force than $Seps1^{+/+}$ littermates, as indicated by a downward shift in the force frequency curve. However, muscle strength was unaffected by exercise. Furthermore, in $Seps1^{+/+}$ littermates, the exercise protocol had no significant effect of TA muscle fatigability and force recovery. Whereas, in $Seps1^{+/-}$ mice the exercise protocol appeared to compromise muscle endurance, such that fatigueability was increased and force recovery was reduced, when compared to exercised $Seps1^{+/+}$ and sedentary $Seps1^{+/-}$ mice. In contrast, sedentary $Seps1^{-/-}$ mice TA muscle endurance was compromised when compared with $Seps1^{+/+}$ littermates; however, three bouts of treadmill running stimulated adaptive processes such that fatigueability and force recovery no longer differed from $Seps1^{+/+}$ mice. Although, this improvement in TA muscle endurance *in situ* did was not reflected by improved voluntary treadmill running performance.

The mechanism underpinning the effects of Seps1 on exercise capacity and TA muscle performance, are still under investigation. However, they are not mediated by increased muscle damage and inflammation, as indicate by muscle morphology and histological and circulating inflammation. In summary, our findings confirm that the global deletion of Seps1 compromises exercise capacity and strength of fast twitch muscles. Furthermore the cellular stress responses to short term strenuous exercise differ between $Seps1^{+/-}$ and $Seps1^{-/-}$ mice.

Moghadaszadeh B, Rider BE, Lawlor MW, Childers MK, Grange RW, Gupta K, Boukedes SS, Owen CA, Beggs AH. (2013). Selenoprotein N deficiency in mice is associated with abnormal lung development. *FASEB J* **27**, 1585-99.

Wright CR, Allsopp GL, Addinsall AB, McRae NL, Andrikopoulos S, Stupka N. (2017). A reduction in selenoprotein S amplifies the inflammatory profile of fast-twitch skeletal muscle in the *mdx* dystrophic mouse. *Mediators Inflamm*, 2017 doi: 10.1155/2017/7043429