Role for selenoprotein S (SEPS1) in regulating skeletal muscle contractile function in fast twitch muscles

A. Addinsall,[1] N.L. McRae,[1] C. Wright,[2] S. Andrikopoulos[3] and N. Stupka,[1] [1]School of Medicine, Deakin University, Pigdons Road, Waurn Ponds, VIC 3216, Australia, [2]School of Exercise and Nutrition Sciences Deakin University, Pigdons Road, Waurn Ponds, VIC 3216, Australia and [3]Department of Medicine - Austin Health, The University of Melbourne, 145 Studley Rd, Heidelberg, VIC 3084, Australia.

Selenoprotein S (SEPS1) is an antioxidant protein with oxidoreductase activity against H_2O_2 and is highly expressed in skeletal muscle although its physiological function is poorly characterized. In C2C12 myoblasts, SEPS1 gene suppression using siRNA increased H_2O_2 levels and was associated with a more oxidised cellular redox state. In skeletal muscle, accumulation of reactive oxygen species (ROS), such as H_2O_2, can alter calcium sensitivity and in turn contractile function (Andrade *et al.*, 1998). Using affinity isolation and mass spectrometry, SEPS1 was recently found to interact with diverse range of multiprotein complexes, including Serca2a and CaMK IIδ (Turanov *et al.*, 2014). These findings together with SEPS1's cellular localisation to the plasma membrane and the endoplasmic reticulum (ER) - since the sarcoplasmic reticulum is a specialised ER - led us to develop the hypothesis that SEPS1 is implicated skeletal muscle contractile function.

Here, male global SEPS1 deleted mice were generated by PGK-Cre. The animal studies were approved by the Animal Ethics Committees at the University of Melbourne and Deakin University, in accordance with NH&MRC guidelines. At ~12 weeks of age, $SEPS1^{-/-}$ (N = 10), $SEPS1^{+/-}$ (N = 9) mice and wild type littermates (N = 10) were placed in metabolic cages for 25 hours to determine VO_2, VCO_2 and physical activity (Columbus Instruments). Prior to contractile function testing, mouse body composition was determined (EchoMRI). Mice were then anaesthetized *via* IP injection of medetomidine (0.5 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg), such that they were unresponsive to tactile stimuli. Fast twitch EDL and slow twitch *soleus* muscles were surgically excised and muscle force production at increasing stimulation frequency (force frequency curve), endurance and recovery from fatigue were assessed *in vitro* (Aurora Scientific). Afterwards, mice were humanely euthanised by cervical dislocation and tissues were collected for molecular and histological analysis.

Body weight, lean mass, fat mass, EDL and *soleus* muscle mass were similar between $SEPS1^{-/-}$, $SEPS1^{+/-}$ and wild type mice and no difference in VO_2 and VCO_2 was observed, indicating that global deletion of SEPS1 does not affect body composition or whole body metabolism. However, physical activity, as assessed by total movement, was approximately 20% lower in $SEPS1^{-/-}$ and $SEPS1^{+/-}$ mice compared to wild type littermates ($P<0.04$).

In $SEPS1^{-/-}$ mice there was a downward shift in the force frequency curve compared to $SEPS1^{+/-}$ and wildtype littermates ($P<0.001$ and $P<0.005$, respectively; main effect for genotype GLM ANOVA), indicating that the loss of SEPS1 expression may compromise muscle force (P_o) production. Following 4 min of rest, muscle endurance was assessed by stimulating EDL muscles submaximally at 60 Hz every 5 s for 4 min, and then again at 2 min, 5 min and 10 min post fatigue to assess force recovery. During the 4 min of intermittent contractile activity, EDL muscles from $SEPS1^{-/-}$ and $SEPS1^{+/-}$ produced less force than EDL muscles from wild type littermates ($P<0.001$ and $P<0.007$, respectively; main effect for genotype GLM ANOVA). When compared to wild type littermates, the $SEPS1^{-/-}$ and $SEPS1^{+/-}$ mice also produced approximately 20% less force during recovery from fatigue ($P<0.001$ and $P<0.032$, respectively; main effect for genotype GLM ANOVA). An unexpected observation was that force (P_o) produced at 60 Hz stimulation during the force frequency curve was reduced by 10% compared to the force produced at the start of the fatigue run in $SEPS1^{-/-}$ mice ($P<0.03$; dependent T-test) and $SEPS1^{+/-}$ mice ($P<0.000$; dependent T-test), but not wild type mice.

Thus, the genetic deletion or reduction SEPS1 appears to decrease muscle force production in fast twitch EDL muscles, and this may contribute to the reduction in physical activity observed in the $SEPS1^{+/-}$ and $SEPS1^{-/-}$ mice. Future experiments will investigate how modulating SEPS1 expression affects skeletal muscle contractile function in response to the increase in cellular stress induced by strenuous exercise. Experiments are ongoing to characterize the cellular location of SEPS1 in skeletal muscle and to determine whether expression is fibre type specific.

Andrade FH, Reid MB, Allen DG, Westerblad H. (1998). Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J Physiol* **509,** 565-75.

Turanov AA, Shchedrina VA, Everley RA, Lobanov AV, Yim SH, Marino SM, Gygi SP, Hatfield DL, Gladyshev VN. (2014). Selenoprotein S is involved in maintenance and transport of multiprotein complexes. *Biochem J* **462,** 555-65.