Novel role for Selenoprotein S in regulating skeletal muscle contractile function in fast twitch mouse muscles

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Selenoprotein S (SEPS1) is an antioxidant protein with oxioreductase activity against H_2O_2. In humans, polymorphisms in the SEPS1 promoter have been linked to diseases with a heightened pro-inflammatory state. SEPS1 is also protective against endoplasmic reticulum (ER) stress by facilitating the translocation of misfolded proteins out of the ER lumen for degradation in the cytosol. SEPS1 is highly expressed in skeletal muscle, yet its physiological function *in vivo* is poorly characterised. In cultured C2C12 myoblasts, SEPS1 gene suppression using siRNA increased H_2O_2 levels and was associated with a more oxidised cellular redox state, as indicated by the oxidised to reduced glutathione (GSH:GSSG) ratio.

Skeletal muscle contractile function and metabolism are sensitive to oxidative, ER and inflammatory stress, and our aim was to characterise the effects of reduced SEPS1 expression on whole body metabolism and skeletal muscle function. Male global SEPS1 deleted mice were generated by PGK-Cre and fed a standard chow diet. 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, mice were placed in metabolic cages for 25 h (Metabolic Analyser, Columbus instruments) to determine VO_2, VCO_2 and the respiratory exchange rate ratio (RER). The physical activity of the mice was measured during this time (Animal Activity Meter: Opto-Varimex-Mini, Columbus instruments). Prior to contractile function testing, mouse body composition was assessed (ESF-005, EchoMRI), and then mice were anaesthetized *via* intraperitoneal injection of medetomidine (0.6 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg) such that they were unresponsive to tactile stimuli. Fast twitch *extensor digitorum longus* (EDL) and slow twitch *soleus* muscles were surgically excised and muscle force production, endurance and recovery from fatigue were assessed *in vitro* (1300A Whole Mouse Test System, Aurora Scientific) (Stupka *et al.*, 2008). Following functional testing, anaesthetised mice were humanely euthanized by cervical dislocation and the heart and hindlimb muscles were collected for molecular and histological analysis.

Here, we present our preliminary findings on $SEPS1^{+/-}$ mice and wild type littermates (N = 5-6). Hindlimb muscles, specifically the fast twitch tibialis anterior (TA) muscle, of $SEPS1^{+/-}$ had a 61% reduction in SEPS1 mRNA transcripts (P=0.04). Body weight, lean mean mass and fat mass were similar between $SEPS1^{+/-}$ and wild type mice. Physical activity, as assessed by ambulatory movement was 32% lower in $SEPS1^{+/-}$ mice (P=0.02). Although, no difference in VO_2 and VCO_2 was observed. SEPS1 heterozygosity had no effect on EDL or soleus muscle mass, twitch force (P_t), maximal force (P_o) and specific force (sP_o). To assess muscle endurance and force recovery, EDL and soleus muscles were stimulated submaximally at 60 Hz every 5 s for 4 min, and then again at 2 min, 5 min and 10 min post fatigue. During 4 min of contractile activity, EDL muscles from the $SEPS1^{+/-}$ mice fatigued more than those from wild type littermates (P=0.001; main effect – general linear model ANOVA). Furthermore, recovery from fatigue was ~30% worse in EDL muscles from $SEPS1^{+/-}$ mice (P=0.006; main effect – general linear model ANOVA). Whereas in soleus muscles, SEPS1 heterozygosity did not compromise endurance and recovery from fatigue. In fast twitch muscles recovery of force following submaximal contractile activity is modulated by cellular redox state (Lamb & Westerblad, 2011). Thus, reduced SEPS1 expression in EDL muscles may increase cellular stress in response to contractile activity, and this could contribute to the decrease in physical activity observed in the $SEPS1^{+/-}$ mice. In conclusion, these preliminary data suggest a new role for SEPS1 in modulating skeletal muscle contractile function.

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