A reduction in Selenoprotein S (SEPS1) amplifies the inflammatory profile of fast twitch skeletal muscle in the mdx dystrophic mouse

G. Keefe,[1] A. Addinsall,[2] S. Andrikopoulos,[3] N. Stupka[2] and C. Wright,[1] [1]Centre for Physical Activity and Nutrition (C-PAN), School of Exercise and Nutrition Sciences, Deakin University, Waurn Ponds, VIC 3216, Australia, [2]School of Medicine, Deakin University, Waurn Ponds, VIC 3216, Australia and [3]Department of Medicine - Austin Health, The University of Melbourne, Heidelberg, VIC 3084, Australia.

Selenoprotein S (SEPS1) is hypothesized to protect against inflammatory stress. Polymorphisms in the human SEPS1 gene are associated with elevated TNFα, IL-1β and IL-6 gene expression (Curran *et al.*, 2005), and are associated with inflammatory related diseases such as type II diabetes (Walder *et al.*, 2002; Gao *et al.*, 2004) and obesity (Olsson *et al.*, 2011). In several cell culture lines, the siRNA knockdown of SEPS1 upregulates inflammatory cytokines (Kim *et al.*, 2007; Zeng *et al.*, 2008; Fradejas *et al.*, 2011). SEPS1 is highly expressed in human skeletal muscle, however the role of SEPS1 in mediating skeletal muscle inflammation is unknown.

Here we investigated the effects of a reduction in SEPS1 on the inflammatory profile of the *mdx* dystrophic mouse, a murine model of Duchenne Muscular Dystrophy. Male C57BL6 mice with a global heterozygous deletion of SEPS1 were generated using the Cre-LoxP system, and crossbred with female *mdx* mice to produce F1 male *mdx* mice with a heterozygous deletion of SEPS1 (*mdx*-SEPS1$^{-/+}$) and *mdx* male controls. Body composition was measured between six and 12 weeks, after which the EDL and *soleus* muscles underwent *in situ* analysis of force, fatigability and force recovery. Briefly, mice were anaesthetized *via* IP 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 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 (Aurora Scientific; n = 11-13 mice per group). Anaesthetized mice were then humanely euthanized by cervical dislocation. Following muscle function testing, the EDL and *soleus* were collected for mRNA and protein expression. All procedures were carried out with full approval from the Deakin University Animal Ethics Committee (AEC#: G29/2014).

Western blotting revealed that global heterozygous deletion of SEPS1 in the *mdx* mouse caused a robust reduction (51 %) in SEPS1 protein in the fast twitch muscle *tibialis anterior* (P=0.034), however there were no differences in EDL or *soleus* muscle mass; therefore a reduction in SEPS1 is probably not affecting skeletal muscle growth. SEPS1 knockdown mice had a 2.4 fold increase in monocyte chemoattractant protein 1 (MCP-1) mRNA (P=0.044), a two-fold increase in macrophage marker F4/80 mRNA (P=0.047) and a trend for elevated transforming growth factor beta 1 (TGF-β1) mRNA in the fast-twitch EDL muscle (P=0.056). Unlike the EDL muscles, mRNA levels of pro-inflammatory cytokines and F4/80 were not altered in the *soleus* muscles of *mdx*-SEPS1$^{-/+}$ mice. This suggests that reduced SEPS1 expression may elevate inflammation in fast-twitch, but not slow twitch muscles of *mdx* mice. The effects of these changes in gene expression on inflammatory cell infiltration, degeneration and regeneration need to be assessed using histological and immunohistochemical techniques. It should be noted that in EDL and *soleus* muscles of 12 week old *mdx* mice, the genetic reduction of SEPS1 had no effects on muscle force, fatigability or recovery *in vitro*. Further morphometric analyses of skeletal muscle in *mdx*-SEPS1$^{-/+}$ mice are required. Therefore, these preliminary data suggest that genetic reduction of SEPS1 in the *mdx* mouse appears to exacerbate the inflammatory profile of EDL muscles without affecting muscle function.

Curran JE, Jowett JB, Elliott KS, Gao Y, Gluschenko K, Wang J, Abel Azim DM, Cai G, Mahaney MC, Comuzzie AG, Dyer TD, Walder KR, Zimmet P, MacCluer JW, Collier GR, Kissebah AH, Blangero J. (2005). *Nature Genet* **37**, 1234-41.

Fradejas N, Del Carmen Serrano−Pérez ZM, Tranque P, Calvo S. (2011). *Glia* **59(6)**, 959-72.

Gao Y, Feng HC, Walder K, Bolton K, Sunderland T, Bishara N, Quick M, Kantham L, Collier GR. (2004). *FEBS Lett* **563**, 185-90.

Kim K-H, Gao Y, Walder K, Collier GR, Skelton J, Kissebah AH. (2007). *Biochem Biophys Res Commun* **354**, 127-32.

Olsson M, Olsson B, Jacobson P, Thelle DS, Björkegren J, Walley A, Froguel P, Carlsson LM, Sjöholm K (2011). *Metabolism* **60**, 114-20.

Walder K, Kantham L, McMillan JS, Trevaskis J, Kerr L, De Silva A, Sunderland T, Godde N, Gao Y, Bishara N, Windmill K, Tenne-Brown J, Augert G, Zimmet PZ, Collier GR. (2002). *Diabetes* **51**, 1859-66.

Zeng J, Du S, Zhou J, Huang K. (2008). *Arch Biochem Bioph* **478**, 1-6.