An essential role for mitochondrial phosphatidylethanolamine synthesis in regulating skeletal muscle and mitochondrial structure

A. Selathurai, G.M. Kowalski and C.R. Bruce, Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC 3125, Australia. (Introduced by Victoria Foletta)

Phosphatidylethanolamine (PE) is the second most abundant phospholipid in mammals. PE is synthesized *via* two pathways, cytidine diphosphate (CDP)-ethanolamine pathway located in the endoplasmic reticulum and the phosphatidylserine decarboxylase (PSD) pathway in the mitochondria. Recently, we have identified that PE derived from the CDP-ethanolamine pathway plays an important role in regulating skeletal muscle and mitochondrial function (Selathurai *et al.*, 2015). However, very little is known about the functional significance of the PSD pathway in skeletal muscle. Therefore, we aimed to examine the role of the PSD pathway in skeletal muscle.

We have combined the use of AAV vectors with shRNA technology to knockdown the expression of PSD in adult muscle. A rAAV6 vector containing an shRNA sequence against PSD (rAAV6:PSD shRNA) was injected into the right *tibialis anterior* (TA) muscle of 8 week old C57Bl/6 mice. The left TA was injected with an rAAV6 vector containing a scrambled shRNA sequence (rAAV6:scrambled). Eight weeks after AAV injection, the TA muscles were excised , weighed and prepared for histology or biochemical analysis.

The rAAV6:PSD shRNA vector reduced PSD protein levels by 60-70% and caused 40% reduction in TA mass. Histochemical analysis revealed that PSD-deficient muscle exhibit a dramatic increase in the number of centrally located nuclei as well as a reduction in myofibre size and number. This was associated with marked changes in mitochondrial morphology. The intensity of staining for succinate dehydrogenease, NADH-tetrazolium reductase and cytochrome oxidase was also reduced by PSD knockdown.

These findings suggest that PE derived from the PSD pathway is essential for maintaining mitochondrial integrity in skeletal muscle. Furthermore, we demonstrated that disrupting the mitochondrial membrane lipid environment can severely impact skeletal muscle structure and function.