The role of mitochondrial derived phosphatidylethanolamine in regulating skeletal muscle structure and function

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Phosphatidylethanolamine (PE) is the second most abundant phospholipid in mammals. The major route for PE synthesis in mammals is believed to be the cytidine diphosphate (CDP)-ethanolamine (Kennedy) pathway which is located in the endoplasmic reticulum. In recent studies we have identified that PE derived from the CDP-ethanolamine pathway plays an important role in regulating skeletal muscle function and mitochondrial biology (Selathurai *et al.*, 2015). Indeed, eliminating the CDP-ethanolamine pathway in skeletal muscle resulted in a reduction in muscle mass while also stimulating mitochondrial biogenesis which was associated with enhanced exercise capacity (Selathurai *et al.*, 2015). In addition to the CDP- ethanolamine pathway, mammalian cells have an alternate pathway for PE synthesis which involves the decarboxylation of phosphotidylserine *via* reactions catalyzed by phosphotidylserine decarboxylase (PSD). This is known as the PSD pathway and is localised to the mitochondria. Mitochondrial membranes are enriched with PE. In fact, PE is more abundant in mitochondria than in any other organelle, comprising ~30% of mitochondrial phospholipid. Very little is known about the functional significance of the PSD pathway in skeletal muscle, and in mammalian biology generally. Therefore, given that we have previously shown that PE derived from the CDP-ethanolamine pathway is essential for muscle function, we wished to examine whether mitochondrial-derived PE would also play a role in regulating skeletal muscle structure and function.

Deciphering the role of the PSD pathway in mammalian biology has been complicated by the fact PSD knockout mice are embryonic lethal (Steenbergen *et al.*, 2005). We have overcome this by combining the use of AAV vectors with shRNA technology to knockdown the expression of PSD in adult muscle. Eight week old C57Bl/6 mice were anaesthetized with isoflurane and the right *tibialis anterior* (TA) muscle was injected with an rAAV6 vector containing an shRNA sequence against PSD (rAAV6:PSD shRNA). The left TA was injected with an rAAV6 vector containing a scrambled shRNA sequence (rAAV6:scrambled). All procedures were approved by the Deakin University Animal Ethics Committee. Eight weeks after AAV injection, mice were anaesthetized with isoflurane, the TA muscles were excised, weighed and prepared for histological or biochemical analysis. Mice were then killed by cardiac excision whilst still anaesthetized.

The rAAV6:PSD shRNA vector reduced PSD protein levels by around 60-70% ($P<0.05$) which caused a 40% reduction in TA mass (51.5±0.9 *vs* 32.1±0.6 mg for rAAV6:scrambled and rAAV6:PSD shRNA respectively; $P<0.001$). Histochemical analysis revealed that PSD-deficient muscle exhibited 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, with knockdown of PSD causing accumulation of enlarged, swollen mitochondria that exhibited severe cristae disruption. The intensity of staining for succinate dehydrogenase, 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 demonstrate that disrupting the mitochondrial membrane lipid environment can severely impact on skeletal muscle structure and function.

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