Phospholipids and skeletal muscle function

C.R. Bruce, School of Exercise and Nutrition Sciences, Centre for Physical Activity and Nutrition Research, Deakin University, 221 Burwood Highway, VIC 3125, Australia.

Phospholipids are essential structural components of cell membranes. However, it is now apparent that they also have regulatory roles in processes such as cell division, cell signalling and autophagy. It is therefore not surprising that phospholipids could play an important role in regulating skeletal muscle physiology. Indeed, disrupting the synthesis of phosphatidylcholine, the most abundant phospholipid in mammals, causes the development of rostrocaudal muscular dystrophy in mice (Sher *et al.*, 2006). Furthermore, recent studies have identified that individuals with a congenital muscular dystrophy have mutations in the gene encoding choline kinase β, an enzyme involved in phosphatidylcholine synthesis *via* the CDP-choline pathway, which causes abnormal mitochondrial morphology and early onset muscle wasting (Mitsuhashi *et al.*, 2011; Cabrera-Serrano *et al.*, 2015). While emerging evidence suggests that phosphatidylcholine is important for regulating normal muscle function, little is known about the role of phosphatidylethanolamine. Phosphatidylethanolamine is the second most abundant phospholipid in mammals, comprising 20-50% of total phospholipid content.

Phosphatidylethanolamine is synthesized by two spatially distinct pathways: (i) the CDP-ethanolamine branch of the Kennedy pathway which is located in the endoplasmic reticulum, with the rate limiting enzyme being CTP:phosphoethanolamine cytidylyltransferase (ECT); and (ii) decarboxylation of phosphatidylserine *via* reactions catalysed by phosphatidylserine decarboxylase (PSD) which is localised to the inner mitochondrial membrane. We have begun to examine the role of both of these pathways in skeletal muscle physiology.

To understand the significance of the CDP-ethanolamine pathway in muscle, we have generated a muscle-specific ECT knockout mouse. While the muscle-specific ECT knockout mice are outwardly indistinguishable from littermate controls, they display an intriguing muscle phenotype having less lean mass which is attributed to a reduction in muscle size. Investigations using stable isotope tracer methodology are currently underway to examine the basis of this reduction in muscle mass. To examine whether this muscle phenotype is specifically due to loss of ECT, we have used an AAV vector expressing ECT to re-introduce ECT into muscle of the knockout mice. Restoring ECT activity normalised muscle mass, demonstrating that phenotype of the muscle-specific ECT knockout mice is specifically mediated by a deficiency of ECT.

To examine the role of mitochondrial-derived phosphatidylethanolamine, we have combined the use of AAV vectors with shRNA technology to knockdown PSD expression in skeletal muscle of adult mice. PSD knockdown was found to have marked effects, resulting in a 40% reduction in muscle mass which was associated with an accumulation of severely damaged mitochondria that exhibited cristae disruption. In addition, PSD-deficient muscle was characterized by an increase in centrally located nuclei as well as evidence of fibre branching. We are currently focused on examining the mechanisms mediating this effect.

Thus, our studies reveal a new and intriguing role for phosphatidylethanolamine derived from both the CDP-ethanolamine and PSD pathways in regulating skeletal muscle function and mitochondrial biology.

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