Mechanisms of SR Ca^{2+} ATPase dysfunction in muscle wasting disorders

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The absence of dystrophin renders muscle fibres fragile, prone to damage and results in loss of Ca^{2+} homeostasis that can activate degenerative pathways leading to myofibre death. It is widely accepted that dystrophic muscles have an elevated cytosolic [Ca^{2+}] both at rest and following damaging contractions. We and others have shown that improving the expression and activity of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA; the primary Ca^{2+} control mechanism in muscle) can increase Ca^{2+} sequestration into the sarcoplasmic reticulum (SR), reduce cytosolic [Ca^{2+}] and ameliorate the dystrophic pathology (Goonasekera *et al*., 2011; Gehrig *et al*., 2012). Although it is well established that membrane lipid composition affects the activity of membrane proteins such as SERCA, comparatively little research has investigated the therapeutic modulation of membrane lipid composition for disorders mediated by reduced SERCA activity, such as heart disease and the muscular dystrophies.

SERCA activity is influenced by bilayer thickness, head group, and membrane fluidity but also by the lipid class composition of the bilayer. Phosphatidylethanolamine (PE) content, which represents 16% and 27% of lipids in the skeletal and cardiac SR membranes, respectively, increases maximal SERCA activity (Hunter *et al*., 1999). Similarly, phosphatidylinositol (PI) and phosphatidylserine (PS) influence SERCA activity, as does cholesterol content in the SR (and ER more generally). An increased ratio of phosphatidylcholine (PC) to PE in the ER membrane has been causally implicated in reduced SERCA activity in liver tissue from obese mice (Fu *et al*., 2011) which was reversed by viral modulation of the activity of phosphatidylethanolamine N-methyltransferase (Pemt), a critical enzyme involved in PE to PC conversion.

Skeletal muscle fatty acid synthase modulates SERCA activity and muscle function *via* effects on SR membrane phospholipids (Paran *et al*., 2015). In diaphragm muscles, lipogenesis was reduced 50% in *mdx* dystrophic mice compared with wild type controls with SR phospholipidome analysis revealing elevated PC and PC:PE in the *mdx* mice. In preliminary studies we found large magnitude changes in expression of individual lipid classes in SR membranes from dystrophic *mdx* and *dko* mice compared to WT control, including PI, PC and PE classes, cardiolipin, modified ceramide and diacylglycerol. There was a high similarity in expression within the *mdx* and *dko* mice suggesting a similar mechanism may be responsible for the aberrant lipid expression in both models of muscular dystrophy. We confirmed a significant reduction in the maximal SERCA activity in SR membranes from mixed hindlimb and diaphragm muscles of *mdx* and *dko* mice compared to WT mice and found a significant correlation between SR membrane PC content and maximal SERCA activity. This implies that by increasing the PC content in the SR of dystrophic mice we could increase SERCA activity and thus ameliorate the dystrophic pathology. Recent studies have confirmed this in primary myocytes with the restoration of the muscle lipogenic machinery normalising PC:PE and rescuing SERCA function in *mdx* myotubes (Paran *et al*., 2015).

We are investigating approaches to modulate the enzymes involved in controlling lipid metabolism and lipid species conversion in order to normalize the aberrant lipid composition and thus enhance SERCA activity and slow pathological progression in muscular dystrophy. We are also examining whether aberrant SR lipid composition is characteristic of multiple muscle wasting disorders, perhaps contributing to the altered SERCA function in cancer cachexia.

Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, & Hotamisligil GS (2011). *Nature* **473**, 528-31.

Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA & Lynch GS (2012). *Nature* **484**, 394-398.

Goonasekera SA, Lam CK, Millay DP, Sargent MA, Hajjar RJ, Kranias EG & Molkentin JD (2011). *J Clin Investn* **121**, 1044-1052.

Hunter GW, Negash S, Squier TC (1999). *Biochemistry* **38**, 1356-1364.

Paran CW, Zou K, Ferrara PJ, Song H, Turk J & Funai K (2015). *Biochim Biophys Acta* **1851**, 1530-1538.

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