Glycogen dysregulation and cardiomyocyte dysfunction in a rat model of type 1 diabetes

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Diabetic cardiomyopathy is characterized by early metabolic changes linked with disturbances in cardiac glucose handling and increased glycogen storage (Laughlin *et al.*, 1990) (Nakao *et al.*, 1993). Recent work from our group has built on early anecdotal observations of glycogen accumulation in the diabetic heart and revealed a significant relationship between cardiac glycogen and measures of cardiac relaxation, suggesting that glycogen accumulation may be a major contributor to diastolic dysfunction in diabetic heart pathology. The causes and consequences of glycogen accumulation in the diabetic heart remain to be fully understood. The aim of this study was to investigate the effects of glycogen handling on cardiac function, and examine the pathways of glycogen metabolism in the diabetic heart.

All animal experiments were performed at the University of Auckland and approved by the relevant institutional Animal Ethics Committee in accordance with the guidelines and regulations of Code of Practice for the Care and Use of Animals for Scientific Purposes. All animals were anesthetized by isoflurane followed by cervical dislocation. Cardiomyocytes isolated from male type 1 diabetic Sprague Dawley rats (streptozotocin (STZ), 55mg/kg i.p., 8 weeks duration) were apportioned to glycogen analysis or loaded with Fura2 Ca^{2+} fluorescent dye for assessment of Ca^{2+} handling (ratiometric signal F360: 380nm, IonOptix). A separate cohort of STZ rats were injected with an inhibitor of autophagosome-lysosome fusion (chloroquine (CQ), 50mg/kg i.p) 4 hours prior to tissue collection. Glycogen was measured using an amyloglucosidase enzymatic assay. Western blots were performed on STZ cardiac tissue probing for glycogen synthase and phosphorylase.

A 2-fold increase in glycogen in the diabetic heart was observed ($P<0.05$). A right shift in the cell shortening-Ca^{2+} phase-loops was observed in isolated diabetic cardiomyocytes, with a significantly higher level of Ca^{2+} at 50% cell length relaxation (EC50) (1.41 ± 0.03 *vs* 1.52 ± 0.03, $P<0.05$). Correlation of glycogen content with EC50 values showed a significant positive relationship ($r=0.6025$, $P<0.05$). Glycogen synthase phosphorylation, an inhibitory action of the enzyme, was significantly higher in the STZ rat compared to the control (1.0 ± 0.15 *vs* 6.70 ± 0.74, $P<0.05$). Glycogen phosphorylase phosphorylation which activates the enzyme was also significantly elevated in the STZ rat compared to control (1.0 ± 0.10 *vs* 1.70 ± 0.16, $P<0.05$). CQ-induced lysosomal blockade increased cardiac glycogen by 45% in control rats ($P<0.05$) but not STZ rats.

This study is the first to show that glycogen accumulation in the diabetic heart affects myofilament Ca^{2+} function and thus could explain impairments in relaxation. In addition the findings that glycogen synthase is inhibited and phosphorylase is activated suggest that cytosolic regulation of glycogen content is not sufficient to counteract the high levels of glycogen present in the diabetic heart. The finding from this study that lysosomal glycogen breakdown is disturbed in the diabetic heart provide a novel mechanism to explain glycogen overload in the diabetic heart. Further investigation into the role of glycogen autophagy (‘glycophagy’) in the diabetic heart is now warranted.

Laughlin MR, Petit WA, Jr., Shulman RG & Barrett EJ. (1990). Measurement of myocardial glycogen synthesis in diabetic and fasted rats. *Am J Physiol* **258**, E184-190.

Nakao M, Matsubara T & Sakamoto N. (1993). Effects of diabetes on cardiac glycogen metabolism in rats. *Heart and Vessels* **8**, 171-175.