STBD1 regulation of myocardial glycogen content

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Myocardial glycogen accumulation is associated with severe functional defects including atrial arrhythmias and diastolic dysfunction. Glycogen storage diseases provide evidence that autophagic processes are crucial in regulating cardiomyocyte glycogen levels. Glycophagy, a glycogen specific autophagy, has been recently described in the heart and a deficit in this pathway may contribute to cardiac glycogen excess (Mellor *et al.*, 2014; Reichelt *et al.*, 2013). Starch-binding domain-containing protein 1 (STBD1) is a key glycophagy protein, shown to bind to glycogen and may facilitate breakdown in the autophagosome in COSM9 cell line (Jiang, Wells and Roach, 2011). The aim of this study was to investigate the role of STBD1 in regulating myocardial glycogen content and the downstream effects on cardiac function, with a specific focus on diastolic dysfunction.

A CRISPR model of heterozygous STBD1 knockout (STBD1-KO) was produced and animals were euthanised at post-natal day 2, and also at 10wks and 30wks of age (pentobarbital, 20mg/kg dose). Echocardiography was conducted to assess measurements of diastolic function (E/E' and mitral valve deceleration time) and systolic function (ejection fraction and fractional shortening) for adult animals 1 week prior. Allele deletion was verified by conventional PCR. Ventricles were homogenised for glycogen content *via* enzymatic assay.

Post-natal day 2 heterozygous STDB1-KO exhibited lower cardiac glycogen content compared to STBD1-WT animals (17.73%, $P<0.05$) with no observable systemic or structural deficits. At 10 weeks, there were no differences in cardiac glycogen content. The STBD1-KO animals did have smaller hearts relative to body weight (vs WT, 7.65%, $P<0.05$), associated with a higher E/E' (vs WT, 24.6%, $P<0.05$), lower mitral valve deceleration time (vs WT 37.15%, $P<0.001$) and no change in ejection fraction and fraction shortening. Interestingly, at 30 weeks, cardiac glycogen content was lower in the KO animals (vs WT, 29.58%, $P<0.05$).

This study provides first evidence of STBD1 as a key protein mediating glycogen content *in vivo.* In addition, a decrease in cardiac glycogen may be associated with diastolic, but not systolic function. An understanding of the mechanisms mediated myocardial glycogen content may provide novel therapeutic targets in metabolic diseases affecting the heart.

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