

Insulin-induced glycogen accumulation is associated with increased glycogen-autophagy in hyperglycaemic cardiomyocytes

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Diabetic cardiac pathology is characterised by metabolic dysregulation and diastolic dysfunction. Glycogen accumulates in the diabetic myocardium, however the impact of excess cardiac glycogen in diabetes is not yet understood. We have previously determined that insulin resistance is associated with an increase in autophagy-induced cell loss (Mellor *et al.*, 2010). In non-cardiac tissue, it has been demonstrated that there is a glycogen-specific autophagic pathway ('glycophagy', Jiang *et al.*, 2010). We have recently demonstrated the occurrence of cardiac glycophagy in an *in vivo* setting (Reichelt *et al.*, 2013), and we hypothesise that this may contribute to the modulation of cardiac glycogen content. This *in vitro* study examines the effect of insulin on glycogen handling and autophagy regulation in cardiomyocytes exposed to high glucose.

Neonatal rat ventricular myocytes (NRVMs) were isolated and cultured in control (5 mM) and high (30mM) glucose media with 0, 1 and 10 nM insulin with or without bafilomycin. Western blotting and immunohistochemistry techniques were used to determine protein expression and localisation of macroautophagy and glycophagy markers in cardiomyocytes. Insulin signalling intermediates were also evaluated by western blot. Glycogen content was assessed by periodic acid-schiff staining of methanol-fixed NRVMs and enzymatic assay of cell lysates.

Insulin-induced activation of the insulin signalling pathway was evident with 5 mM glucose conditions (2.3 fold increased in pAkt:Akt expression, $p < 0.05$), but exposure to high glucose attenuated this effect. Accumulation of glycogen was observed in response to insulin in hyperglycaemic NRVMs, linked with an increase in autophagic 'tagging' of glycogen by starchbinding protein domain-1 (STBD1, 50% increase, $p < 0.05$). Interestingly, the lysosomal inhibitor, bafilomycin, induced accumulation of macroautophagy (microtubule-associated light chain 3B-II (LC3B-II, 2 fold increase, $p < 0.05$) and p62 (1.3 fold increase, $p < 0.05$) but not glycophagy (STBD1 and GABARAPL1) markers. Immunohistochemical investigation confirmed that glycophagic and macroautophagic pathways are distinct in cardiomyocytes.

This study provides the first evidence that glycogen content and glycogen-specific autophagic signalling are regulated by extracellular insulin and glucose in cardiomyocytes. Dysregulation of glycophagy may contribute to glycogen accumulation in diabetic cardiac pathology. Further investigation is now required to elucidate the *in vivo* role of glycogen autophagy.

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