Dissecting the molecular pathways of glucose mishandling and glycogen disturbance in the diabetic heart

K.M. Mellor,[1,2] U. Varma,[2] C.L. Curl[2] and L.M.D. Delbridge,[2] [1]Department of Physiology, University of Auckland, Auckland 1023, New Zealand and [2]Department of Physiology, University of Melbourne, Melbourne, VIC 3010, Australia.

Background: Diabetic cardiomyopathy is characterized by diastolic dysfunction but the underlying mechanisms remain unknown. Dysregulated cardiomyocyte glucose handling and metabolism in diabetes plays an important role in the cardiac pathology. The aim of this study was to investigate the role of disturbances in cardiac glucose handling and AMPK (key metabolic regulator) signalling in the development of diabetic cardiomyopathy.

Methods: Mice with diet-induced obesity and insulin resistance (high fat diet (HFD) *vs* AIN93G control, 15 weeks) were evaluated for systemic glucose tolerance, cardiac function (echocardiography under isoflurane anaesthesia), cardiac glycogen (enzymatic assay after animals were euthanased with pentobarbitone) and protein expression (immunoblot). Neonatal rat ventricular myocytes (NRVMs) were cultured in normal (5mM) or high (30mM) glucose for 24 hours with AMPK agonist treatment (AICAR, 1mM, 30 min).

Results: HFD-fed mice exhibited increased body weight (27%, $P<0.01$) and decreased glucose tolerance. Diastolic dysfunction in HFD mice was evidenced by increased ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/E′, 39%, $P<0.05$) and positively correlated with body weight and glucose intolerance. Glycogen content was increased in the hearts of HFD mice (46%, $P<0.05$), associated with decreased phosphorylation of AMPK (Thr172, 23%, $P<0,05$). No changes in glycogen synthase or phosphorylase protein expression/phosphorylation were observed. NRVMs exposed to high glucose similarly exhibited increased glycogen content (36%, $P<0.05$) which was unaffected by AMPK activation *via* AICAR. siRNA and CRISPR-Cas9 knockout mouse experiments are underway to provide new mechanistic data on glycogen regulation in diabetic cardiomyocytes.

Conclusions: This study is the first to show that diastolic dysfunction correlates with cardiomyocyte glycogen overload in an obese, insulin resistant setting. These findings identify that disturbance of cardiomyocyte glucose storage plays a role in diabetic cardiopathology, and is not attenuated by activation of AMPK. Further investigation of alternative signalling pathways in this setting is now warranted.