The role of protein kinase D in cardiac glucose metabolism in diabetic cardiomyopathy

M.C. Renton,[1] K.A. De Jong,[2] L.G. Hall,[2] T. Connor,[2] S.D. Martin,[2] G.M. Kowalski,[1] C.R. Bruce,[1] C.S. Shaw,[1] S.L. McGee[2] and K.F. Howlett,[1] [1]Instutute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC 3216, Australia and [2]Metabolic Research Unit (MRU), School of Medicine, Deakin University, Geelong, VIC 3216, Australia.

Diabetic cardiomyopathy (DCM) is a specific cardiovascular disease with no known cure and is a leading cause of death in individuals with diabetes mellitus. DCM is progressive, with early signs observed prior to the onset of overt type 2 diabetes mellitus (T2DM) (De Jong *et al*, 2017) and is linked to impairments in glucose oxidation. Protein Kinase D (PKD) inactivation protects against myocardial dysfunction in animal models of early stage DCM, but the mechanisms involved are unknown (Venardos *et al*., 2015). This study aimed to determine whether genetic PKD inactivation protects against myocardial dysfunction by increasing cardiac glucose metabolism. Cardiac-specific dominant negative PKD (DN PKD) and wild type (WT) mice were fed either a high fat diet (HFD) or control diet (CHOW) (n=5-9 mice per group) for 20 weeks. Genetic inactivation of PKD preserved cardiac function, assessed by echocardiography, in mice fed a HFD. An [U-^{13}C] oral glucose challenge was performed, and after 60 minutes mice were killed and hearts collected and frozen for later analysis. *In vivo* cardiac glucose flux was assessed through [U-^{13}C] targeted metabolomics, using gas chromatography mass spectrometry, to measure [U-^{13}C] labelling of key glucose metabolism intermediates. Data showed no significant differences in overall glucose flux into TCA cycle intermediates between DN PKD and WT mice fed either the HFD or CHOW diet. To confirm these findings, the phosphorylation of pyruvate dehydrogenase (PDH), a major rate-limiting step in glucose metabolism, was measured, which is a proxy measure of PDH activity. No differences were found in either PDH total protein or phosphorylation levels. However, when phosphorylated PDH was adjusted for total PDH protein levels, a significant (P<0.05) genotype effect was found, with greater phosphorylated PDH protein observed in DN PKD mice compared to WT mice. No genotype effect was found for PDK4 gene expression; however, a significant (P<0.05) diet effect was found with reduced PDK4 gene expression found in HFD mice compared to CHOW mice. These findings suggest that changes in cardiac glucose metabolism are unlikely to explain the cardio-protective effects of PKD inactivation in mice fed a HFD.

De Jong KA, Czeczor JK, Sithara S, McEwen K, Lopaschuk GD, Appelbe A, Cukier K, Kotowicz M, McGee SL. (2017). Obesity and type 2 diabetes have additive effects on left ventricular remodelling in normotensive patients-a cross sectional study. *Cardiovasc Diabetol* **16** 21. doi: 10.1186/s12933-017-0504-z

Venardos K, De Jong KA, Elkamie M, Connor T, McGee SL. (2015). The PKD inhibitor CID755673 enhances cardiac function in diabetic db/db mice. *PloS one* **10(3)**:e0120934. doi: 10.1371/journal.pone.0120934