The link between *in vivo* diastolic function and mechanical stiffness in intact rat cardiomyocytes

A.J.A. Raaijmakers, J.V. Janssens, J.R. Bell, C.L. Curl and L.M.D. Delbridge, Department of Physiology, University of Melbourne, VIC 3010, Australia.

Background: Diastolic heart failure is characterized by ventricular stiffness and inadequate filling of the ventricles. In addition to collagen deposition, evidence suggests that cardiomyocyte specific intrinsic pathologies may also underlie ventricular stiffness, but the mechanisms are not well understood. The aim of this study was to evaluate intact cellular stiffness properties in a diabetic model of diastolic dysfunction and also to relate these findings directly to the *in vivo* functional properties of the origin hearts.

Methods: Echocardiography was performed in 15-week male Sprague Dawley rats +/-8weeks Streptozotocin (STZ; 55mg/kg) treatment (GE Vivid 9). Isolated cardiomyocytes were prepared by collagenase dissociation. Glass fibres were attached (MyoTak) at the cell longitudinal surface, and paced cardiomyocytes (1Hz, 2.0mM Ca^{2+}, 37°C) were serially stretched (0-11.2%, piezomotor). Sarcomere length/shortening, force development and intracellular Ca^{2+}transients (Fura-2AM, 5μM) were simultaneously measured (Myostretcher, IonOptix).

Results: Hearts from diabetic animals displayed diastolic dysfunction (E/E′ 14.8±0.9 *vs* 10.4±0.6; STZ *vs* Control; mean±SEM; $P<0.05$). When cardiomyocytes were uniformly stretched, measured force was significantly higher in myocytes from the STZ hearts compared to Control (6.88±0.3 *vs* 5.55±0.4 μN; N=4&5, n=4&8, $P<0.05$). A positive correlation between diastolic function measured in intact hearts (MV DecT) and diastolic force from intact cardiomyocytes ($r^2=0.72$, $P=0.006$) was identified, establishing a link between whole heart and single cell diastolic function.

Conclusion: Evaluation of intact cardiomyocyte force development, using glass fibre attachment provides a measure of cardiomyocyte stiffness which can be related to *in vivo* cardiac function. Specifically, in diabetes, cardiac diastolic dysfunction is associated with increased cardiomyocyte mechanical stiffness.