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Revealing the molecular basis of the specific cardiomyopathy associated with the type 2 diabetes

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Performed at BL40XU

Research purpose and background

With an estimated 60% of Australians over the age of 25 now being overweight or obese, the current incidence of type 2 diabetes has increased. Similar statistics are reported in many other countries. In the industrialized world the mortality due to stroke, myocardial infarction and heart failure as a result of diabetes is escalating with this incidence of obesity. The pathology of diabetes however is complex. Nevertheless, it has been established that independent of hypertension and coronary artery disease associated with atherosclerosis (plaque formation in vessel lumens) the development of insulin resistance leads to a specific cardiomyopathy. Insulin stimulates the heart to utilise glucose as a metabolic fuel. The diabetic disease state is characterized by impaired heart muscle relaxation, and a progressive increase in left ventricle (LV) muscle stiffness, fibrosis and eventually congestive heart failure. Prior to the development of overt diabetes many researchers report that systemic lipid overload occurs with obesity. One current hypothesis for the cause of diabetic myopathy that needs further investigation is the hypothesis that inappropriate cardiac fuel utilization (lipid and glucose) causes toxic accumulation of lipids in cardiac myocytes in the insulin-resistant state [1, 2].

We therefore, proposed to determine if the rate of cross-bridge detachment during LV relaxation is directly affected by the extent of cardiac lipid accumulation in the early stages of diabetes induced by streptozotocin treatment (STZ, 65 mg/kg bolus intraperitoneal injection). As in previous experiments we examined cross-bridge dynamics *in vivo* in the left ventricle of Sprague-Dawley rats (age 10 weeks) in both the front and rear walls of the same hearts.

Experimental / analytical method

All rats were treated with STZ (in Citrate Buffer pH 4.6, 100 mM) or vehicle 3 weeks prior to experiments. Open-chest anaesthetized rats were prepared and small angle X-ray diffraction recordings made simultaneous with LV pressure-volume loops as described in earlier reports (2002A~2006A). All experiments were performed at the 40XU beamline with an X-ray energy of 15 keV (attenuated by an aluminium bar), using a Hamamatsu image intensifier and a time resolution of 15 ms for the fast CCD camera.

Mass transfer of myosin was calculated from the decrease in integrated intensity ratio ($I_{1,0}/I_{1,1}$) between end-diastole and end-systole. Myofilament spacing was determined as the distance between first order 1,0 reflections ($d_{1,0}$, nm). In this experimental protocol we investigated muscle contraction mechanisms in the beating heart during under baseline conditions, then during an infusion of dobutamine (beta-adrenoceptor agonist) and finally during volume loading.

Research results

In the 6 shifts available we recorded diffraction patterns from 7 rats. STZ treated rats had elevated glucose levels (12-21 mmol/L, n = 4) and depressed heart rates (200-300 bpm) in comparison to vehicle treated rats (<10 mmol/L, n = 3). Cross-bridge cycling in the STZ rat hearts was significantly altered in the diabetic state. Interestingly, we found in 3 of the 4 STZ hearts that there was a transmural gradient of dysfunction in the cycling of intensity ratio (Figure 1). In the normal beating heart we have previously shown that the diastolic intensity ratio is between 2.0 and 3.0 (left panels) [3], and during systolic contractions the intensity ratio decrease is ~1.0-1.5 (shift in proximity of myosin towards actin). However, in the 3 STZ hearts only the surface

muscle layer (epicardium) demonstrated normal cross-bridge formation (upper right panel). At progressively deeper layers within the heart wall (subepicardium and endocardial layers) diastolic intensity ratio was elevated, and in fact, higher than that reported for resting muscle or arrested hearts (3.0 – 3.5). In general, the 1,1 reflection was still detectable, but intensity was greatly reduced. In 2 of the STZ hearts a weak increase in contractility and heart rate was recorded. In these hearts diastolic intensity ratio was reduced, but remained higher than the vehicle treated rats. This suggests that myosin heads are prevented from occupying close proximity or a weak-binding state during relaxation and filling phases of the cardiac cycle. Nonetheless, STZ hearts did not show any obvious reduction in $d_{1,0}$ spacing changes during contractions in comparison to the controls, except in the deeper endocardial layers.

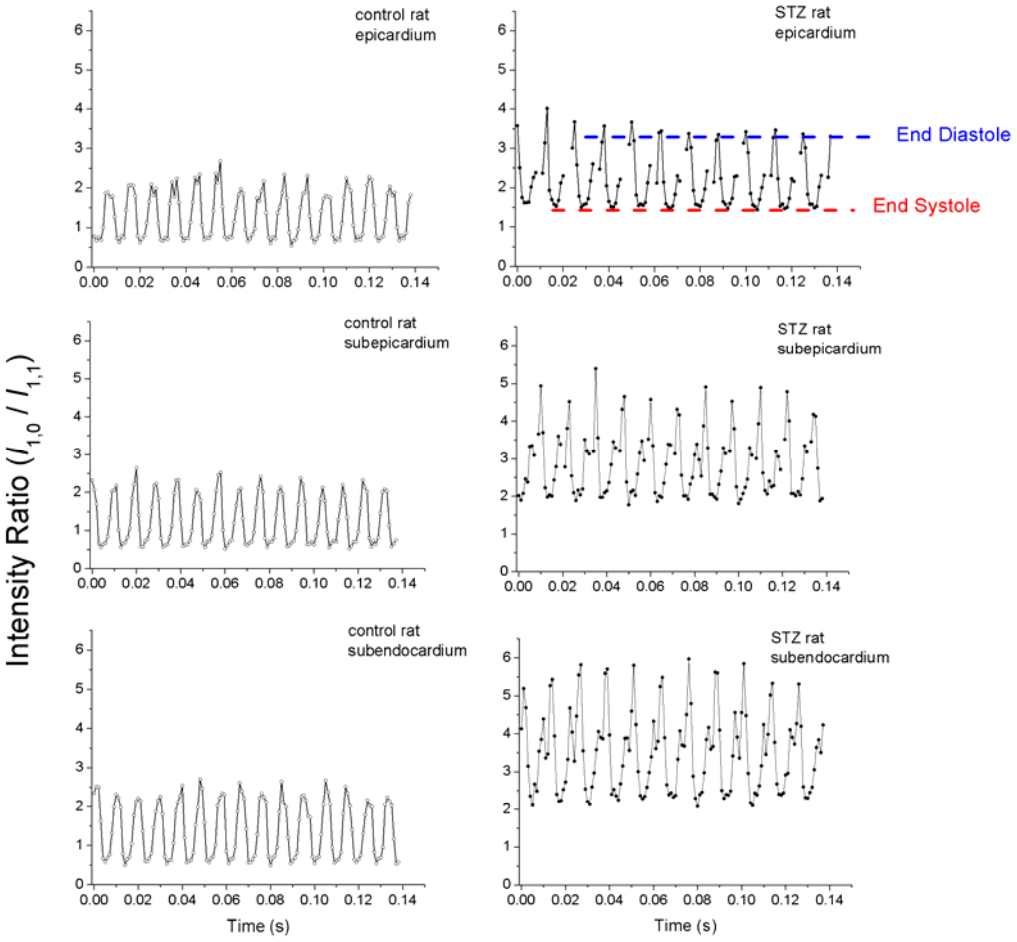

Figure1 shows the cyclic changes in intensity ratio of two rat hearts during spontaneous beating *in situ* for a vehicle (control) and STZ treated animal respectively. The panels presented for both rats show the transfer of myosin heads to actin filaments recorded in a localised region of the heart wall in the epicardium (outer surface, *top panels*) and increasingly deeper intermediate layers towards the innermost endocardium (not shown). The highest intensity ratios are typically recorded during muscle relaxation (diastolic phase of cardiac cycle) and lowest during force development (systole).

Current and future issues / challenges

Our findings are consistent with other studies in that, muscle function in the diabetic rat hearts

was more severely affected in the endocardial layer. Further studies are required to support our findings. In the near future we plan to investigate if the abnormal cross-bridge cycling found in this study correlates with ultrastructural changes in the myocardium. At the same time it will be important to determine if the elevated intensity ratios observed in the cardiac muscle of diabetic rats persists in the arrested heart, which would provide evidence to suggest that the physical and or chemical environment of the myofibrillar lattice is permanently altered in the diabetic state.

References

[1] Bertrand L, Horman S, Beauloye C, Vanoverschelde J-L. Insulin signalling in the heart. Cardiovascular Research 79:238-248, 2008.
[2] Chess DJ, Stanley WC. Role of diet and fuel overabundance in the development and progression of heart failure. Cardiovascular Research 79:269-278, 2008.
[3] Pearson JT, Shirai M, Tsuchimochi H, Schwenke DO, Ishida T, Kangawa K, Suga H, Yagi N: Effects of sustained length dependent activation on in situ cross-bridge dynamics in rat hearts. Biophysics Journal 93:4319-29, 2007.

Status of publication and patent

Additional experiments were required in 2009A to complete this study and to confirm the repeatability of these findings. This we were able to do. A manuscript in now in preparation for submission shortly.

Keywords and annotations

non crystalline small angle X-ray scattering – using X-ray diffraction principles to investigate the electron density of molecules that do not have a crystal lattice structure, but do have periodic organization of molecules, example muscle myofilaments.

interfilament spacing – in the case of muscle, the spacing between 1,0 reflections in the diffraction patterns is equivalent to the average separation between the myosin filaments in the actin-myosin lattice. Since myocytes maintain a constant cell volume the lattice separation of myosin filaments changes in inverse proportion to myocyte cell length and is an important determinant of cross-bridge kinetics.

epicardium and endocardium – the mammalian heart is comprised of two muscle layers of different embryological origin, the outer epicardium and innermost endocardium. An intermediate layer separates these two layers. The three muscle layers differ in their metabolism, stress and strain relations and therefore their vulnerability to disease.