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## Mechanisms underlying the impaired contractility of diabetic cardiomyopathy

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### Abstract

Cardiac dysfunction is a well-known consequence of diabetes, with sustained hyperglycaemia leading to the development of a cardiomyopathy that is independent of cardiovascular disease or hypertension. Animal models of diabetes are commonly used to study the pathophysiology of diabetic cardiomyopathy, with the hope that increased knowledge will lead ultimately to better therapeutic strategies being developed. At physiological temperature, left ventricular trabeculae isolated from the streptozotocin rat model of type 1 diabetes showed decreased stress and prolonged relaxation, but with no evidence that decreased contractility was a result of altered myocardial  $Ca^{2+}$  handling. Although sarcoplasmic reticulum (SR)  $Ca^{2+}$  reuptake appeared slower in diabetic trabeculae, it was offset by an increase in action-potential duration, thereby maintaining SR  $Ca^{2+}$  content and favouring increased contraction force. Frequency analysis of t-tubule distribution by confocal imaging of ventricular tissue labeled with wheat germ agglutinin or ryanodine receptor antibodies showed a reduced T-power for diabetic tissue, but the differences were minor in comparison to other models of heart failure.

The contractile dysfunction appeared to be the result of disrupted F-actin in conjunction with the increased type I collagen, with decreased myofilament  $Ca^{2+}$  sensitivity contributing to the slowed relaxation.

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**Key words:** Diabetic cardiomyopathy; Heart failure; Contractility; T-tubules; Excitation-contraction coupling; Calcium homeostasis

**Core tip:** Diabetic patients develop a cardiomyopathy that is independent of vascular disease, and is thought to develop as a direct result of the prolonged hyperglycaemia. Animal models of diabetes can help us understand the cellular mechanisms that lead ultimately to contractile dysfunction of diabetic cardiomyopathy. The streptozotocin rat model of type 1 diabetes has slowed  $Ca^{2+}$  transients and twitch force kinetics, with reduced myofilament  $Ca^{2+}$  sensitivity. Myocytes are decreased in volume in diabetic hearts, with reduced and disrupted F-actin, and type 1 collagen is increased. Together, these changes all contribute to the reduced contractility of diabetic cardiomyopathy.

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### INTRODUCTION

Patients with diabetes develop a cardiomyopathy that is independent of coronary artery disease and hypertension<sup>[1]</sup>, and contributes to the increased mortality and morbidity of the disease<sup>[2,3]</sup>. The mechanisms that lead to

development of the diabetic cardiomyopathy are poorly understood, although they appear to be a direct result of cellular damage from the hyperglycaemia. The early stages of the cardiomyopathy are associated with reduced diastolic function, with 27%-70% of asymptomatic diabetic patients showing some form of diastolic abnormality<sup>[4-6]</sup>. Later this progresses to include systolic dysfunction and heart failure<sup>[7,8]</sup>. Diabetes manifests in two forms, both of which are a result of abnormal glucose metabolism. Type I diabetes usually has its onset early in life and is characterized by insufficient insulin production, whereas type II diabetes has its origin downstream of insulin binding to its receptor, and is therefore known as insulin-resistant diabetes. Diabetic cardiomyopathy develops in both type I and type II forms of the disease<sup>[9,10]</sup>.

Although the heart contains many different cell types, it is the cardiac myocytes that perform the work that enables the heart to function as a pump. With each cardiac cycle, the myocytes experience rapid changes in intracellular ion concentrations that are crucial to the hearts inotropy, lusitropy, and energy metabolism. This review will outline the ultrastructural and functional changes that contribute to the impaired contraction and relaxation characteristic of diabetic cardiomyopathy.

## MECHANISMS CONTRIBUTING TO DIABETIC CARDIOMYOPATHY

### **Streptozotocin rat model of diabetes**

Animal models have frequently been used in research into the cellular mechanisms associated with diabetes<sup>[11]</sup>, with the insulin-deficient streptozotocin rat (STZ) commonly studied. Type-1 diabetes in humans is characterized by the destruction of the pancreatic  $\beta$ -cells, as occurs in the STZ. Streptozotocin is a naturally occurring glucose analog that is particularly toxic to the insulin-producing beta cells of the pancreatic islets. The chemical is transported into cells *via* the glucose transporter-2 (GLUT-2)<sup>[12]</sup>. Since the pancreatic beta cells have high levels of GLUT-2, they accumulate streptozotocin in large quantities, resulting in their destruction and the onset of a diabetic state. Rats treated with a single dose of streptozotocin (60 mg/kg) rapidly develop biochemical and functional myocardial abnormalities. They exhibit increased water consumption (180 mL/d compared to 43 mL/d for sham-injected control) and elevated plasma glucose levels (31 mmol/L compared to 4 mmol/L for control) that are sustained. Isolated cardiac muscle preparations from diabetic rats 8 wk post-injection show depressed contractility, diminished compliance and decreased inotropic drug responses<sup>[13]</sup>. Abnormalities in contraction and metabolism have been reported both *in vivo* and *in vitro* in the STZ diabetic rat model, reflecting changes at the cardiac myocyte level as a result of the sustained hyperglycaemia. The STZ rat has proved an invaluable model for investigation of the pathogenesis of type 1 diabetes and its complications, and in the development of potential new treatments for the disease<sup>[14-16]</sup>.

### **The reduced contractility of diabetic hearts**

Contraction in cardiac muscle is brought about by an increase in the myocyte intracellular  $\text{Ca}^{2+}$  concentration (the “ $\text{Ca}^{2+}$  transient”). Propagation of the action potential across the surface sarcolemma and throughout the transverse tubule system (t-tubules) opens voltage-gated L-type  $\text{Ca}^{2+}$  channels causing a synchronised influx of  $\text{Ca}^{2+}$  into the myocytes (the “ $\text{Ca}^{2+}$  current”). This  $\text{Ca}^{2+}$  current then triggers release of  $\text{Ca}^{2+}$  from the junctional region of the sarcoplasmic reticulum (SR) *via* the ryanodine receptors (RyRs) in a process termed “ $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release”<sup>[17,18]</sup>. In this way the intracellular  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  is rapidly increased to approximately 10 times the resting level.  $\text{Ca}^{2+}$  then diffuses to the contractile proteins where it binds to troponin C, initiating cross-bridge cycling and force development. Excitation-contraction coupling has therefore been a major focus of those investigating the cellular mechanisms that underlie the reduced contractility of failing hearts.

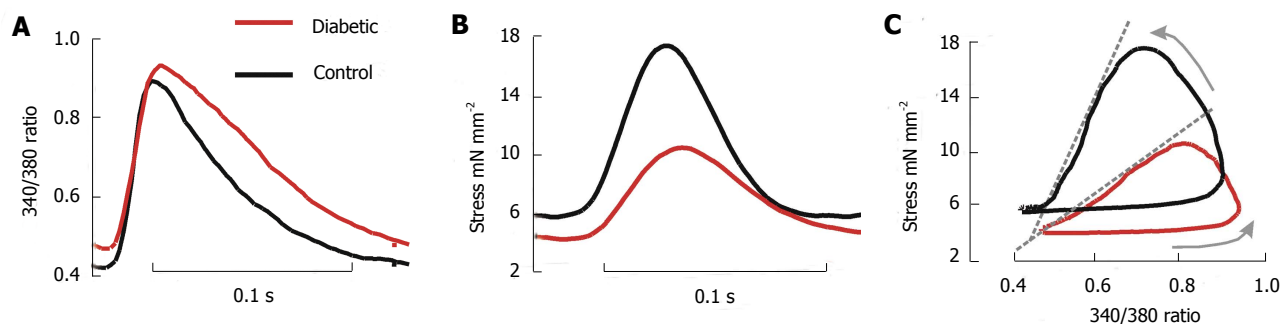
### **Intracellular calcium transients in diabetic hearts**

Measurements carried out on multicellular trabeculae isolated from the left ventricle under near physiological conditions (1.5 mmol/L  $[\text{Ca}^{2+}]_o$ , 37 °C and 5 Hz) showed trabeculae from diabetic rats had depressed contractility with prolonged contraction and relaxation in comparison to their controls, consistent with other studies<sup>[19-21]</sup>.

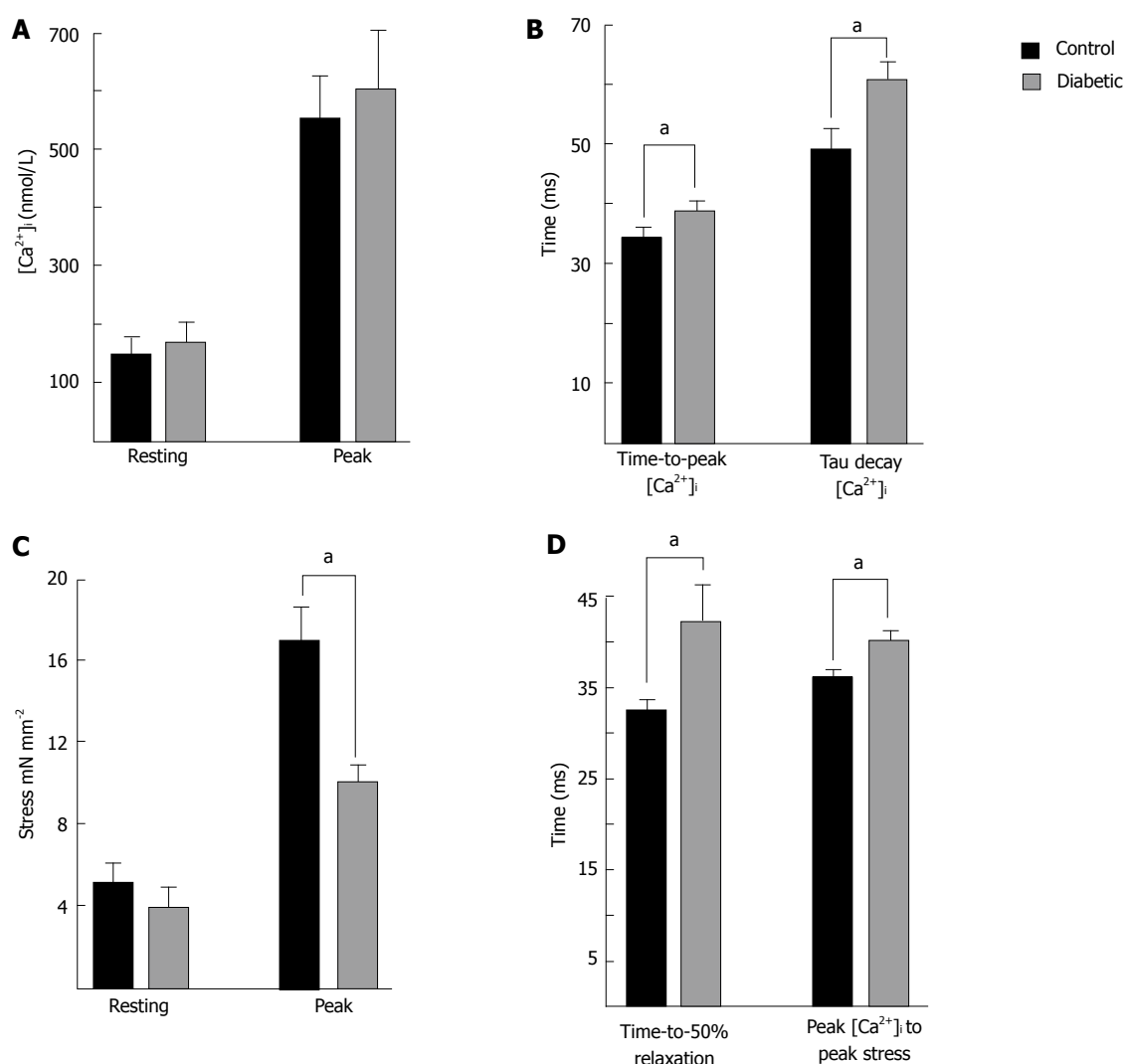
An alteration of intracellular  $\text{Ca}^{2+}$  homeostasis has previously been suggested as underlying the diabetic cardiac dysfunction (for review see<sup>[22]</sup>) although, as noted, results are often contradictory. While some of these discrepancies might be attributable to the extent of disease progression (diabetic stage) and experimental conditions, very few studies have examined the  $[\text{Ca}^{2+}]_i$  control of contractility under near-physiological temperatures and rates of stimulation. Our study showed that diabetic rats had an unchanged resting  $[\text{Ca}^{2+}]_i$  level and amplitude of  $\text{Ca}^{2+}$  transient, despite a reduced contractility<sup>[23]</sup>. Averaged  $\text{Ca}^{2+}$  transients and isometric twitches at 5 Hz stimulation are shown in Figure 1 for trabeculae from control (solid line) and diabetic (dotted line) rats, superimposed for comparison. Figure 1C shows the  $[\text{Ca}^{2+}]_i$ -stress phase plot, with a right shifted relaxation phase for diabetic trabeculae which suggests diminished myofibrillar  $\text{Ca}^{2+}$  sensitivity.

Figure 2 shows averaged data from trabeculae at 5 Hz stimulation and at 37 °C. Diabetic rats had prolonged time-to-peak  $[\text{Ca}^{2+}]_i$  and a prolonged time constant of  $\text{Ca}^{2+}$  transient decay, consistent with some other reports<sup>[20,24-26]</sup>. The slower kinetics of  $\text{Ca}^{2+}$  transient would contribute to the prolonged time course of cardiac contraction and relaxation in diabetic rats, but it is unclear if the reduced rate of the decay in the  $\text{Ca}^{2+}$  transient is sufficient to explain the slowed mechanical relaxation.

Our study showed that contractility was reduced in trabeculae from diabetic hearts, even when peak  $[\text{Ca}^{2+}]_i$  was matched between diabetic and control trabeculae by altering stimulation rate<sup>[23]</sup>, suggesting that altered  $[\text{Ca}^{2+}]_i$  handling was not the primary mechanism of contractile



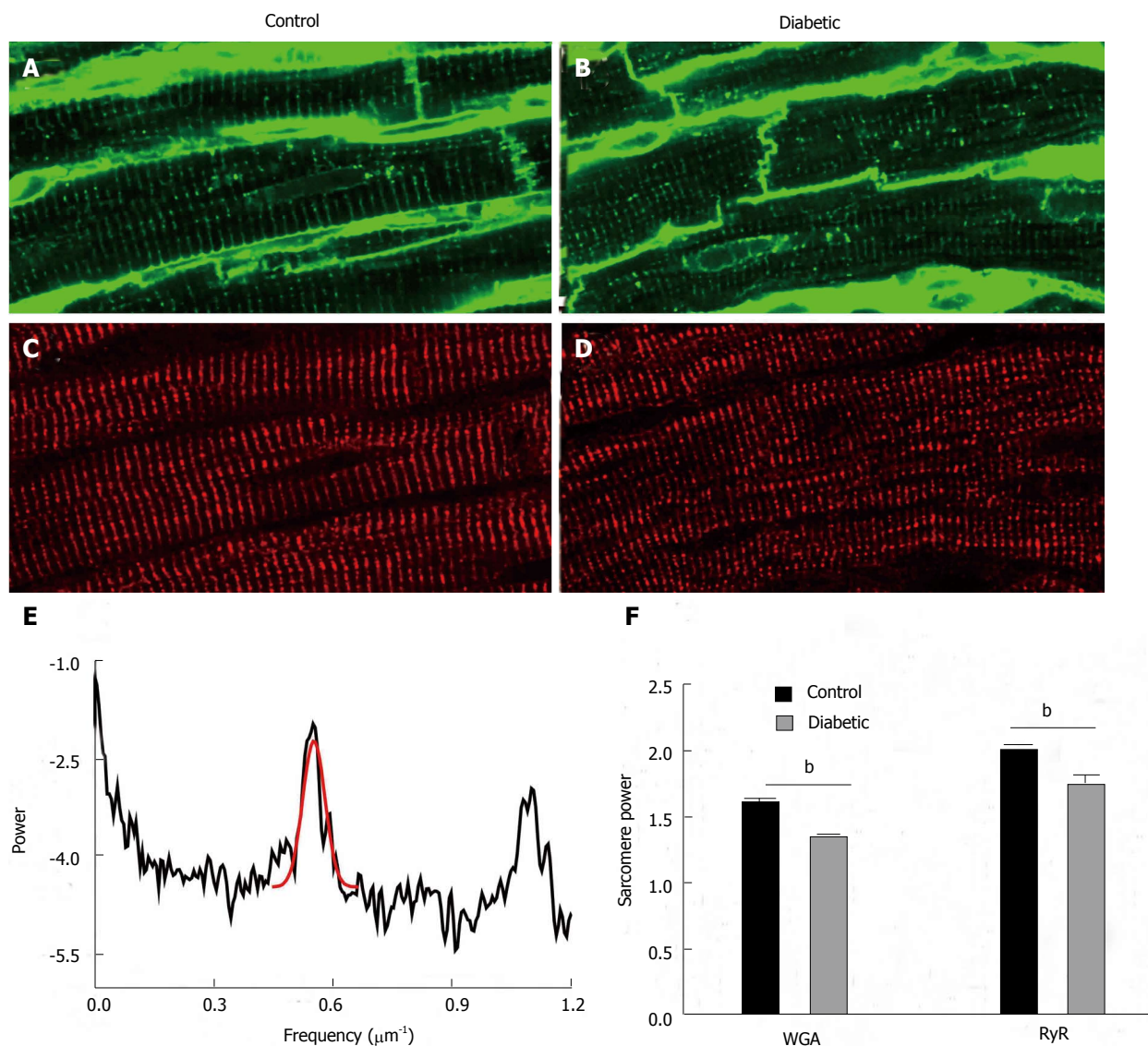
**Figure 1 Average intracellular  $\text{Ca}^{2+}$  transients and isometric stress.** Data were recorded from left ventricular trabeculae of diabetic (red lines) and control (black lines) hearts at 5 Hz, 37 °C, and 1.5 mmol  $[\text{Ca}^{2+}]_o$ , 7 trabeculae per group. A:  $\text{Ca}^{2+}$  transient (340/380 fluorescence ratio); B: Stress; C: Phase plots of the relationship between fluorescence and stress. The arrows indicate the direction of time, and the dashed grey lines accentuate the slope of the relaxation component. (Modified from Zhang *et al*<sup>[23]</sup>).



**Figure 2 Summary of intracellular  $\text{Ca}^{2+}$  and isometric stress parameters.** Data were recorded from left ventricular trabeculae at 37 °C, 5 Hz stimulation frequency, and 1.5 mmol  $[\text{Ca}^{2+}]_o$ . Data are mean  $\pm$  SE 8 wk post injection for control ( $n = 7$ ) and diabetic ( $n = 8$ ). A: Shows resting and peak  $[\text{Ca}^{2+}]_i$ . The  $\text{Ca}^{2+}$  transients were prolonged in diabetic trabeculae; B: Shows the time to reach peak  $[\text{Ca}^{2+}]_i$ , and the time constant of the  $\text{Ca}^{2+}$  transient decay; C: Shows no difference in resting stress, but peak stress was reduced in diabetic trabeculae; D: Shows the time to 50% relaxation of stress was prolonged in diabetic, as was the time from the peak of the  $\text{Ca}^{2+}$  transient to the peak of the twitch. <sup>a</sup> $P < 0.05$ , diabetic vs control.

dysfunction. The mechanical relaxation was intrinsically slower in diabetic rat hearts, which was exacerbated by the reduced rate of decrease of  $[\text{Ca}^{2+}]_i$ . In support of this

idea, Figure 2D shows that the interval between the time-to-peak  $[\text{Ca}^{2+}]_i$  and the time-to-peak stress in diabetic rats was increased in comparison to control.



**Figure 3 Structural changes in proteins associated with excitation-contraction coupling.** Transverse tubules were visualised by labelling with wheat germ agglutinin in (A) control and (B) diabetic tissue. The same tissue sections were dual labelled with antibodies against ryanodine receptors (RyR) in (C) control and (D) diabetic tissue. The periodicity or regularity of labelling was assessed using a fast Fourier transform. An example of this analysis is shown in (E) which is the plot of the FFT in control myocyte labelled with RyR. The peak associated with sarcomeric periodicity (approximately  $0.55 \mu\text{m}^{-1}$ ) is fitted with a Gaussian in red. The height of this peak is used as a metric to assess the regularity of sarcomere labelling termed "sarcomere" power. (F) This shows the mean sarcomere power for both wheat germ agglutinin and ryanodine receptor labelling from 18 cells from 3 control animals and 18 cells from 3 diabetic animals. Both wheat germ agglutinin and ryanodine receptor sarcomere power were modestly but highly significantly reduced in cells from diabetic hearts (Bonferroni corrected *t* test, <sup>b</sup>*P* < 0.01, diabetic vs control.).

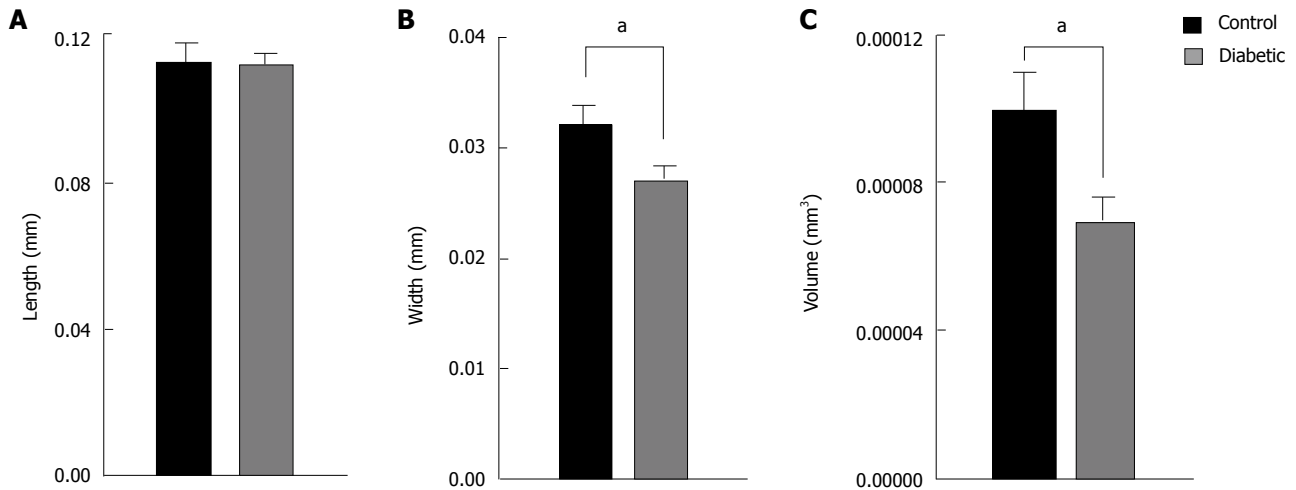
Analysis of electrocardiogram (ECG) in lightly anesthetized diabetic rats prior to experimentation showed that the normalized QT interval was prolonged, implying the cardiac action potential was slower<sup>[23]</sup>. This would contribute to the prolonged  $\text{Ca}^{2+}$  transients observed in diabetes, but cannot explain the observed  $\text{Ca}^{2+}$  transient changes in full. Logarithmic plots of  $\text{Ca}^{2+}$  transients from control and diabetic trabeculae in Zhang *et al.*<sup>[23]</sup> (2008) show that the linear portion of the  $\text{Ca}^{2+}$  fluorescence decay was delayed in trabeculae from diabetic hearts, consistent with the increase in the time-to-50% repolarization of the ventricular action potential reported in their study. Prolonged depolarization during the plateau phase of the action potential will lead also to increased L-type  $\text{Ca}^{2+}$  influx, although this was not shown in the Zhang *et al.*<sup>[23]</sup>

(2008) study. Frequently studies have reported changes in SERCA protein expression in explanation of observed changes to the time course of the  $\text{Ca}^{2+}$  transients<sup>[27,28]</sup>, but decreased SERCA activity and/or expression may only contribute in part to the prolonged  $\text{Ca}^{2+}$  transient decay. Action potential duration is also important in determining the duration of the  $\text{Ca}^{2+}$  transient, and therefore the SR  $\text{Ca}^{2+}$  load, which in turn determines SR  $\text{Ca}^{2+}$  release *via* the RyRs<sup>[29]</sup>. ECG measurements in insulin-treated type 1 diabetic patients also show abnormal repolarization with the reports of increased QT interval and increased QT dispersion<sup>[30]</sup>.

### T-tubule system structure

The t-tubules are an important component of the excita-





**Figure 4** Average dimensions of isolated ventricular myocytes from diabetic and control rat hearts. Cell length (A) was not different between diabetic ( $n = 35$ ) and control ( $n = 19$ ) hearts, whereas cell width (B) and cell volume (C) was reduced. <sup>a</sup> $P < 0.05$ , diabetic vs control.

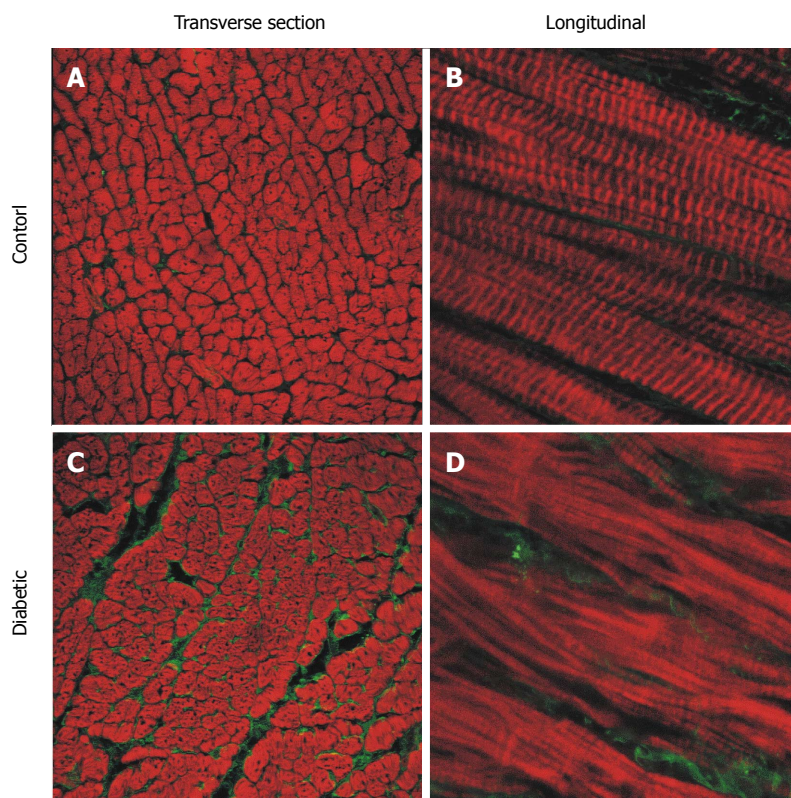
tion-contraction coupling system in cardiac myocytes<sup>[31]</sup>. T-tubules are an extension of the sarcolemma that project transversely into the interior of the cell adjacent to the z-line, although numerous axial connections between sarcomeres are observed<sup>[32]</sup>. This structure facilitates synchronous contraction by conducting the action potential deep within the myocyte and triggering  $Ca^{2+}$  release from the SR in regions located away from the cell surface. There is evidence that loss of normal transverse tubule structure is a key feature of both animal<sup>[33,34]</sup> and human heart failure<sup>[35,36]</sup>. Frequency analysis of t-tubule distribution at the z-line has been used to quantify the structural changes in t-system labelling of myocytes from rodents at different stages of heart failure<sup>[33]</sup>. This analysis exploits the periodic nature of t-tubule distribution at the z-line of sarcomere. By converting t-tubule images into the frequency domain with a fast Fourier transform, a peak associated with sarcomere spacing of  $2 \mu m$  is observed in the power spectrum<sup>[37,38]</sup>. In failing myocytes the periodic pattern of t-tubule labelling is disrupted resulting in reduced sarcomere peak. This peak is termed “T-power” and provides a useful metric to quantify t-tubule structure.

Currently there is lack of comparable data for changes in t-tubules in the diabetic heart. To address this gap in knowledge we have used confocal laser scanning microscopy to examine the labelling of the t-tubules [wheat germ agglutinin (WGA)] and the ryanodine receptors (RyR), in the hearts of STZ rats with end stage heart failure as shown in Figure 3. Analysis of this labelling in the frequency domain has shown a significant but surprisingly modest decrease in T-power (or sarcomere power) in the t-tubule system of diabetic myocytes. A similar analysis of RyR labelling showed a comparable decrease in sarcomere power in diabetic myocytes. Visual inspection of the labelling in Figure 3 shows that both the structure of t-system (WGA) and the SR (RyR) are largely intact in myocytes from diabetic rat hearts, which is consistent with the comparatively normal calcium transients measured in the cardiac trabeculae of this animal

model<sup>[23]</sup>. This contrasts with the dramatic loss of the t-system structure reported in non-diabetic animal heart failure. For example the t-system is dramatically remodelled in spontaneously hypertensive rat, while the labelling of RyR is largely intact<sup>[34,38]</sup>. A similar situation is seen in non-diabetic human heart failure<sup>[36]</sup>. This may turn out to be a key point of difference between diabetic and other forms of heart failure, and it remains to be seen if a similar pattern of t-system preservation is seen in the diabetic human heart. Alternatively, the lack of obvious changes in the t-tubule distribution of STZ-induced diabetic rat hearts 8 wk post injection may reflect the relatively short duration of the disease. Figure 2B shows an increase in the time-to-peak of the  $Ca^{2+}$  transients in longitudinal section (LV) trabeculae from diabetic hearts, which may reflect changes in excitation-contraction coupling from hyperglycaemia-induced loss of t-tubule structure.

#### Ventricular remodeling of diabetic hearts

Although intracellular  $Ca^{2+}$  cycling is essential to the contraction and relaxation of cardiac myocytes, the extracellular matrix and the myofilaments within the myocytes are essential also. The contractile proteins that make up the myofilaments are the end effectors of excitation-contraction coupling, and their responsiveness to  $Ca^{2+}$  directly determines myocyte contractility (for reviews see<sup>[39,40]</sup>). Changes in the contractile proteins of diabetic hearts have been reported, and are likely to contribute substantially to the observed changes in contraction and relaxation. Figure 2C and D show both reduced contraction (peak stress) and slowed relaxation in LV trabeculae from diabetic rat hearts. The slower time course of contraction in trabeculae from diabetic hearts could be explained, in part, by a shift in the myosin isoenzyme distribution from the faster alpha heavy chain to the beta form as previously reported<sup>[41]</sup> (for review see<sup>[42]</sup>). Changes in other aspects of the contractile protein system have also been described in diabetic hearts. The thin filament regulatory troponin-tropomyosin complex shows decreased  $Ca^{2+}$  sensitivity in skinned<sup>[43,44]</sup> and intact<sup>[16]</sup> cardiac muscle



**Figure 5** Representative confocal images of longitudinal section free wall immuno-labelled for type I collagen (green) and f-actin (red). Sections from the endocardium of control (A and B) and diabetic (C and D) rat hearts. Left hand side panels: Transverse sections from endocardium (25 × objective). Right hand side panels: Longitudinal sections (63 × objective, zoom × 3). (Modified from Zhang *et al.*<sup>[23]</sup>).

preparations. The consequence of reduced  $\text{Ca}^{2+}$  sensitivity is increased force production for any given cytosolic  $\text{Ca}^{2+}$  concentration, favouring force production during systole, but decreasing relaxation which would contribute to diastolic failure.

Ultra-structural analysis by electron microscopy has revealed loss and disorganisation of actin filaments in STZ diabetic hearts<sup>[21]</sup>, which was supported by confocal analysis of phalloidin labelled ventricular tissue with disorganisation and a reduction of f-actin labelling evident<sup>[16,21,23]</sup>. We have also observed that myocyte cell diameter is reduced in the STZ diabetic rat, suggesting that amount of myofilament protein per myocyte is reduced. Figure 4 shows mean  $\pm$  SE data from enzymatically isolated ventricular myocytes from diabetic and control rats. Cell length was not different between groups, but both width and volume were markedly reduced in myocytes from diabetic hearts. Similar changes in f-actin content and myocyte size in the STZ diabetic rat have been reported by Kawaguchi *et al.*<sup>[45]</sup> (1999). Pertinently these authors also identified a decrease in myocyte diameter in the diabetic human heart<sup>[45]</sup>. Changes in myocyte volume have been shown to occur as early as one week after induction of diabetes in the STZ<sup>[46]</sup>. The decreased myocyte volume is evident in the left hand side (LHS) panel of Figure 5C and D where representative tissue from the LV free wall of diabetic rat hearts shows reduced myocyte diameter. It appears then that the diabetic myocytes are atrophied. Ultrastructural changes in mitochondrial morphology have been shown by electron microscopy of diabetic rat heart, with likely consequences not only for myocyte volume but also energy metabolism<sup>[21]</sup>. Proteomic analysis of diabetic rat heart identified multitude

of changes in the mitochondrial proteome<sup>[47]</sup>. The most notable changes are an increase in enzymes involved in long chain fatty acids oxidation and decrease in enzymes involved in catabolism. Metabolism of the diabetic heart is shifted from a mix of carbohydrates and fatty acids for energy supply to relying almost solely on fatty acids, with a resultant increase in the production of oxygen free radical end products<sup>[48]</sup>. Significantly the proteomic analysis also showed changes in proteins involved with oxidative stress, suggesting that impaired energy metabolism might lead to myocytes being unable to meet the energetic needs producing changes in the structure and function of the contractile machinery.

Diabetic cardiomyopathy is also associated with increased stiffness in the left ventricle<sup>[49]</sup>, and a decreased maximum rate-of-rise in developed stress<sup>[23]</sup>, suggesting that cardiac compliance is reduced in diabetic rats. The extracellular matrix in healthy hearts provides a scaffolding that supports the myocytes and other tissue components, enabling the coordinated transduction of force that is necessary for the heart to function as a pump. Collagen is an important component of the extracellular matrix, with type I and type III collagens the most abundant types in ventricular tissue forming 90% of the total collagen content<sup>[50]</sup>. Figure 5 shows type I collagen is increased in diabetic rat hearts, which would contribute to the decreased ventricular compliance, with no change in type III collagen<sup>[23]</sup>. Myocardial echodensity has been reported as increased in asymptomatic diabetic patients, thought to be a result of increased collagen deposition<sup>[51]</sup>. It is proposed that increased echodensity might therefore act as an early indicator of the subsequent development of diabetic cardiomyopathy.

## CONCLUSION

In conclusion, diabetic cardiomyopathy arises as a result of the sustained hyperglycaemia and the damaging effects this has on the heart. Ventricular myocytes from untreated diabetic rat hearts show contractile dysfunction after 8 wk of hyperglycaemia, with prolonged action potential duration, slower Ca<sup>2+</sup> transient decay and reduced myofilament Ca<sup>2+</sup> sensitivity. Gross structural changes to the myocardium are evident at this stage of the disease. Extracellular type 1 collagen is increased, t-tubules are less regular in appearance, and F-actin within myocytes is reduced in content and disrupted in appearance. We conclude that it is these structural changes that are the main contributors to the contractile dysfunction of diabetic cardiomyopathy, along with mitochondrial changes that compromise energy supply. We suggest that consideration should therefore be given in future studies to the contribution of these observed structural changes to the contractile deficit in the diabetic hearts, rather than focusing on myocyte Ca<sup>2+</sup> handling in searching for effective treatments for diabetic cardiomyopathy.

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