Coenzyme Q₁₀ attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the *db/db* mouse model of type 2 diabetes

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Received: 12 October 2011 / Accepted: 16 January 2012 / Published online: 29 February 2012
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Abstract

Aims/hypothesis An increase in the production of reactive oxygen species is commonly thought to contribute to the development of diabetic cardiomyopathy. This study aimed to assess whether administration of the antioxidant coenzyme Q₁₀ would protect the diabetic heart against dysfunction and remodelling, using the *db/db* mouse model of type 2 diabetes. Furthermore, we aimed to compare the efficacy of coenzyme Q₁₀ to that of the ACE inhibitor ramipril.

Methods Six-week-old non-diabetic *db/+* mice and diabetic *db/db* mice received either normal drinking water or water supplemented with coenzyme Q₁₀ for 10 weeks. Endpoint cardiac function was assessed by echocardiography and catheterisation. Ventricular tissue was collected for histology, gene expression and protein analysis.

Results Untreated *db/db* diabetic mice exhibited hyperglycaemia, accompanied by diastolic dysfunction and adverse structural remodelling, including cardiomyocyte hypertrophy, myocardial fibrosis and increased apoptosis. Systemic

lipid peroxidation and myocardial superoxide generation were also elevated in *db/db* mice. Coenzyme Q₁₀ and ramipril treatment reduced superoxide generation, ameliorated diastolic dysfunction and reduced cardiomyocyte hypertrophy and fibrosis in *db/db* mice. Phosphorylation of Akt, although depressed in untreated *db/db* mice, was restored with coenzyme Q₁₀ administration. We postulate that preservation of cardioprotective Akt signalling may be a mechanism by which coenzyme Q₁₀-treated *db/db* mice are protected from pathological cardiac hypertrophy.

Conclusions/interpretation These data demonstrate that coenzyme Q₁₀ attenuates oxidative stress and left ventricular diastolic dysfunction and remodelling in the diabetic heart. Addition of coenzyme Q₁₀ to the current therapy used in diabetic patients with diastolic dysfunction warrants further investigation.

Keywords Antioxidant · Cardiomyopathy · Diabetes · Remodelling · Superoxide

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Abbreviations

ACE-I	ACE inhibitor
AMREP	Alfred medical research and education precinct
DBP	Diastolic blood pressure
E:A	Ratio of peak early to late transmitral blood flow velocities
FS	Fractional shortening
H&E	Haematoxylin and eosin
HW	Heart weight
IGF1R	IGF1 receptor
LV	Left ventricular
LVEDD	LV end-diastolic dimension
LVEDP	LV end-diastolic pressure

LVESD	LV end-systolic dimension
MDA	Malondialdehyde
PI3K[p110 α]	Phosphoinositide-3 kinase p110 α isoform
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SERCA2a	Sarco/endoplasmic reticulum Ca ²⁺ -ATPase
TL	Tibial length

Introduction

The global incidence of diabetes mellitus has dramatically increased throughout the past two decades, firmly establishing the condition as one of the most significant health burdens of the 21st century. By the year 2025, 300 million individuals are projected to have diabetes, with the majority of these suffering from type 2 diabetes [1].

Coronary heart disease and peripheral vascular diseases are among the most common causes of mortality in diabetic individuals; however, a specific cardiomyopathy independent of these complications is also evident [2, 3]. Diabetic cardiomyopathy is typically characterised by left ventricular (LV) dysfunction, with diastolic dysfunction manifesting early in the disease [4]. Structural abnormalities such as LV hypertrophy and increased cardiac fibrosis occur together with the onset of diastolic dysfunction in the diabetic heart [5]. The presence of these characteristics acts as a predictive indicator of mortality in diabetic patients, necessitating the development of new pharmacological targets to treat LV dysfunction and remodelling in the diabetic heart.

Despite the importance of diabetic cardiomyopathy as a clinical entity, the molecular mechanisms potentiating the adverse changes in myocardial function and structure are poorly understood. The development and progression of diabetic complications is frequently attributed to an increase in the generation of reactive oxygen species (ROS), in particular superoxide, within susceptible tissues [6, 7]. The balance between the generation and elimination of ROS via antioxidant degradation is critical in maintaining cardiovascular health. As hyperglycaemia impairs the endogenous antioxidant defence system [8], numerous studies have examined the benefits of antioxidant supplementation in the setting of diabetes [9–11].

Coenzyme Q₁₀, an endogenous component of the mitochondrial respiratory chain, functions as an antioxidant in its reduced form, ubiquinol-10. Coenzyme Q₁₀ has previously been shown to inhibit cardiac oxidative stress [12, 13]. A protective role for coenzyme Q₁₀ has been suggested in settings of heart failure outside diabetes [14–16]. However, the potential of coenzyme Q₁₀ to attenuate the structural and functional defects specifically caused by diabetic cardiomyopathy still remains to be elucidated.

The aim of the present study was to assess the efficacy of the antioxidant coenzyme Q₁₀ in protecting the diabetic heart against adverse changes using the *db/db* mouse model of type 2 diabetes. We hypothesised that the administration of coenzyme Q₁₀ would protect against the impaired LV function and adverse structural changes induced by type 2 diabetes. As ACE inhibitors (ACE-Is) are the current gold standard in minimising cardiovascular risk and hypertension in clinical settings, we also aimed to evaluate the efficacy of coenzyme Q₁₀ compared with the ACE-I ramipril in treating diabetic cardiomyopathy.

Methods

Animal model All animal research was conducted in accordance with the National Health and Medical Research Council of Australia guidelines, and was approved by the Alfred Medical Research and Education Precinct (AMREP) Animal Ethics committee.

Female C57BL/6 *db/+* and *db/db* mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The mice were housed in the AMREP Precinct Animal Centre and maintained under a 12 h light–dark cycle. Age-matched female non-diabetic *db/+* mice and diabetic *db/db* mice received a standard diet and normal drinking water. At 6 weeks of age, the *db/+* and *db/db* mice were allocated either to continue receiving regular water, to receive water supplemented with coenzyme Q₁₀ (10 mg/kg; LiQsorb Liposomal CoQ₁₀, a kind gift from R. K. Chopra, Tishcon Corp, NY, USA, using a dose previously shown to limit the renal complications of diabetes [17]) or to receive water supplemented with ramipril (3 mg kg^{−1} day^{−1}; Sigma Aldrich, St Louis, MO, USA). The mice were followed for a further 10 weeks prior to killing and tissue collection. Blood was collected at the endpoint for assessment of plasma glucose (Austin Pathology Service, Heidelberg, VIC, Australia). Animals with plasma glucose levels exceeding 28 mmol/l were considered diabetic. Blood was also retained for measurement of HbA_{1c} by HPLC (CLC330 GHb Analyser; Primus, Kansas City, MO, USA) [18].

Analysis of LV function in vivo Endpoint LV function and chamber dimensions were assessed via two-dimensional targeted M-mode echocardiography and Doppler echocardiography. At 16 weeks of age, mice were anaesthetised with a cocktail of ketamine, xylazine and atropine (100, 10 and 1.2 mg/kg i.p.), and echocardiography was performed using a Philips (North Ryde, NSW, Australia) iE33 ultrasound machine with a 15 MHz linear array transducer.

Variables measured on M-mode echocardiography included LV end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), LV mass (calculated as

[external LV dimension³–LVEDD]×1.055) and fractional shortening (FS, calculated as [(LVEDD–LVESD)/LVEDD]×100%). Doppler echocardiography allowed assessment of LV diastolic filling (LV E:A – the ratio of peak early, E, and late, A, transmitral blood flow velocities) and deceleration time of early (E) velocity [19].

LV pressure was also measured by cardiac catheterisation. A micromanometer-tipped catheter (1.4 F; Millar Instrument Co., TX, USA) was inserted through the right common carotid artery into the LV and the aorta of an anaesthetised mouse (ketamine 100 mg/kg, xylazine 10 mg/kg, atropine 1.2 mg/kg i.p.) as previously described [19]. Variables derived from catheterisation included systolic blood pressure (SBP) and LV end-diastolic pressure (LVEDP).

Tissue collection and histology Following catheterisation, with the animal still anaesthetised, cardiac puncture was performed to collect approximately 1 ml of blood in a heparinised syringe. The heart and lungs were then excised from the animals and their wet weights recorded. The tibia was removed and used to calculate the ratio of heart weight to tibial length (HW:TL). A portion of the ventricle was cut, fixed and embedded in paraffin for histology. Paraffin-embedded sections were cut and stained with haematoxylin and eosin (H&E) or 0.1% Picrosirius Red and analysed as previously described [19]. The remaining portion of the ventricle was snap-frozen and stored at –80°C.

Levels of apoptosis were assessed in paraffin-embedded ventricular sections using the CardioTACS In Situ Apoptosis Detection Kit (Trevigen, Gaithersburg, MD, USA) [20]. This method detects nuclear DNA fragmentation by using a terminal deoxynucleotidyl transferase enzyme, which incorporates labelled nucleotides onto the free 3' OH ends of DNA fragments. Positively stained apoptotic cells were distinguished by blue staining, while negatively stained cells were counterstained red with Nuclear Fast Red. Apoptotic cells were quantified as a percentage of non-apoptotic cells, and the results were expressed as fold levels detected in untreated *db/+* mouse heart.

Gene expression and protein analysis RNA and protein were extracted from frozen tissue samples as previously described [10]. cDNA was produced from DNase-treated RNA via reverse transcription (Taqman Reverse Transcription reagents; Applied Biosystems, Mulgrave, VIC, Australia) at template concentrations of 20 ng/μl, as previously published [10, 19, 21]. SYBR Green chemistry (Applied Biosystems) was employed to determine expression of both β-myosin heavy chain (*Myh7*) and sarco/endoplasmic reticulum Ca²⁺-ATPase (*SERCA2a*, also known as *Atp2a2*), using the Applied Biosystems ABI Prism 7700 Sequence Detection System. Ribosomal 18S (also known as *Rn18s*) was used as the endogenous control. All primers were

generated using murine-specific sequences derived from Genebank, at predetermined concentrations. The relative fold increases in the expression of the gene of interest compared with untreated non-diabetic mice were calculated using the comparative delta-delta C_t (ΔΔC_t) method [10, 22].

Analysis of protein levels SDS-PAGE and western blotting were employed to measure the protein levels of phosphorylated Akt (Ser473) and total Akt. Both antibodies were purchased from Cell Signaling Technologies (Danvers, MA, USA), and used at a 1:1,000 dilution, according to manufacturer's instructions. Akt protein bands were detected at 60 kDa, and results were analysed using the Image J 1.4 software (NIH, Bethesda, MD, USA) [19].

Detection of lipid peroxidation and superoxide generation The levels of lipid peroxidation in plasma samples were determined using the Malondialdehyde-586 kit (Bioxytech MDA-586, Foster City, CA, USA), as per the manufacturer's instructions. Superoxide generation was quantified using lucigenin (5 μmol/l)-enhanced chemiluminescence in fresh heart tissue, as previously described [10]. Results were normalised against tissue weight, and expressed as relative light units per second per milligram.

Statistical analysis All data are presented as mean±SE (unless otherwise specified). Two-way ANOVAs were performed to identify differences between genotypes (*db/+* and *db/db*) and treatments (untreated, coenzyme Q₁₀-treated, ramipril-treated). Interactions between groups were statistically analysed using the Student's Newman–Keuls post hoc test. A value of *p*<0.05 was considered significant.

Results

Hyperglycaemia is evident in *db/db* mice Untreated, coenzyme Q₁₀-treated and ramipril-treated *db/db* mice all exhibited marked hyperglycaemia compared with relative *db/+* controls in terms of both plasma glucose and HbA_{1c} (Table 1). HbA_{1c} levels were modestly but significantly reduced in coenzyme Q₁₀-treated *db/db* mice vs untreated *db/db* mice. Body weight was greater in *db/db* diabetic animals compared with *db/+* animals; neither coenzyme Q₁₀ nor ramipril treatment noticeably affected body weight in either *db/+* or *db/db* mice compared with untreated littermates (Table 1). Lung weight was no different between *db/+* and *db/db* animals (Table 1) and was unaffected by treatment. HW data are discussed below.

Coenzyme Q₁₀ preserves diastolic function Analysis of end-point cardiac function via M-mode echocardiography

Table 1 Post mortem systemic analysis of untreated, coenzyme Q₁₀-treated and ramipril-treated *db/+* and *db/db* mice

Variable	<i>db/+</i> untreated	<i>db/db</i> untreated	<i>db/+</i> + CoQ	<i>db/db</i> + CoQ	<i>db/+</i> + ramipril	<i>db/db</i> + ramipril
<i>n</i>	11	14	12	12	14	14
Plasma glucose (mmol/l)	10.2±0.7	38.8±2.4*	10.3±0.5	39.4±2.4 [†]	10.8±0.5	37.7±1.4 [‡]
HbA _{1c} (%)	3.8±0.1	12.2±0.8*	3.2±0.2	9.2±0.9 ^{†§}	4.0±0.2	11.1±1.4 [‡]
HbA _{1c} (mmol/mol)	18.0±0.5	110±7*	11.5±0.7	77.0±7.5 ^{†§}	20.2±1.0	97.8±12.2 [‡]
Body weight (g)	23.3±0.6	48.6±2.5*	23.0±0.4	53.1±2.0 [†]	21.8±0.3	49.8±1.6 [‡]
HW (mg)	104±2	127±4*	99±2	127±4 [†]	95±1	109±3 ^{‡§¶}
LW (mg)	140±3	134±4	132±3	138±4	136±3	130±3
TL (mm)	15.8±0.1	15.5±0.1	15.7±0.1	15.6±0.1	15.7±0.1	15.5±0.1
HW:TL (mg/mm)	6.5±0.1	8.3±0.2*	6.6±0.3	7.7±0.3 [†]	6.0±0.1	7.4±0.2 ^{‡§¶}
LW:TL (mg/mm)	8.9±0.2	8.4±0.2	8.6±0.2	8.9±0.2	8.5±0.2	8.6±0.2

**p*<0.05 vs *db/+* untreated, [†]*p*<0.05 vs *db/+* CoQ, [‡]*p*<0.05 vs *db/+* ramipril, [§]*p*<0.05 vs *db/db* untreated, [¶]*p*<0.05 vs *db/db* CoQ

CoQ coenzyme Q₁₀; LW lung weight

revealed no differences in FS (a marker of systolic function) between *db/+* and *db/db* mice, across all treatment groups (Table 2). On Doppler echocardiography, a prolonged deceleration time (indicative of impaired diastolic function) was observed in untreated *db/db* mice compared with *db/+* mice (Fig. 1a). Both coenzyme Q₁₀- and ramipril-treated *db/db* mice exhibited reduced deceleration times compared with untreated *db/db* mice (Fig. 1a). A similar trend for E:A ratio to decrease was seen in untreated *db/db* mice vs *db/+* mice (*p*=0.065; Table 2). Endpoint cardiac catheterisation further confirmed the presence of diastolic dysfunction in untreated *db/db* mice, where LVEDP was significantly higher than in untreated *db/+* mice (Fig. 1b). Coenzyme Q₁₀ and ramipril significantly attenuated this increase in LVEDP in *db/db* mice. On real-time PCR, *Serca2a* expression was depressed in untreated diabetic mice, but preserved with coenzyme Q₁₀ and ramipril treatment (Fig. 1c).

Cardiac fibrosis is reduced in coenzyme Q₁₀-treated diabetic mice On Sirius Red-stained sections, diabetes induced a 1.8-fold increase in collagen deposition in untreated *db/db* mice relative to non-diabetic *db/+* mice (Fig. 2). Comparatively, no differences in collagen deposition were observed between the *db/db* and *db/+* genotypes, in both coenzyme Q₁₀- and ramipril-treated animals (Fig. 2). Diabetes-induced increases in collagen deposition were completely ameliorated by coenzyme Q₁₀ and ramipril treatment.

Coenzyme Q₁₀ limits diabetes-induced cardiomyocyte hypertrophy Untreated *db/db* mice had significantly greater HW than *db/+* controls, when normalised to TL (Table 1). Despite HW:TL being greater in ramipril-treated *db/db* mice than *db/+* controls, ramipril-treated *db/db* mice had comparatively smaller hearts than untreated *db/db* mice; a similar trend was evident in coenzyme Q₁₀-treated *db/db* mice, but this was not significant (*p*=0.1). On M-mode echocardiography, LV

Table 2 Analysis of LV dimensions and function by M-mode and Doppler echocardiography and cardiac catheterisation in anaesthetised mice

Variable	<i>db/+</i> untreated	<i>db/db</i> untreated	<i>db/+</i> + CoQ	<i>db/db</i> + CoQ	<i>db/+</i> + ramipril	<i>db/db</i> + ramipril
<i>n</i>	8	8	10	12	10	9
Heart rate (bpm)	381±10	364±13	379±11	370±12	381±8	360±14
External LV dimension (mm)	4.99±0.04	5.53±0.07*	4.93±0.05	5.28±0.08 [†]	4.91±0.05	5.24±0.11 [‡]
LVEDD (mm)	3.41±0.07	3.84±0.09*	3.27±0.06	3.63±0.10 [†]	3.50±0.06	3.72±0.14
LVEDS (mm)	2.01±0.12	2.04±0.12	1.92±0.11	2.10±0.06	2.06±0.05	2.03±0.08
LV mass:TL (mg/mm)	5.62±0.15	7.77±0.40*	5.57±0.14	6.75±0.28 ^{†§}	5.05±0.20	6.31±0.34 ^{‡§}
LVPW (mm)	0.76±0.03	0.80±0.05	0.77±0.02	0.84±0.03	0.71±0.03	0.82±0.09
FS (%)	40±3	45±2	43±2	44±2	42±1	44±2
E:A ratio	1.73±0.08	1.48±0.05	1.74±0.09	1.65±0.08	1.85±0.09	1.75±0.09

**p*<0.05 vs *db/+* untreated, [†]*p*<0.05 vs *db/+* CoQ, [‡]*p*<0.05 vs *db/+* ramipril, [§]*p*<0.05 vs *db/db* untreated

bpm beats per min; LVPW left ventricular posterior wall thickness

Fig. 1 Coenzyme Q₁₀ preserves diastolic function and *Serca2a* expression. In diabetic mice, coenzyme Q₁₀ and ramipril (**a**) decreased deceleration time, (**b**) reduced LVEDP and (**c**) prevented the diabetes-induced fall in the gene expression of *Serca2a*. $n=8-12/\text{group}$. White bars, *db/+*; black bars, *db/db*. * $p<0.05$ vs *db/+* untreated, *** $p<0.001$ vs *db/+* untreated, † $p<0.05$ vs *db/+* coenzyme Q₁₀, ‡ $p<0.05$ vs *db/+* ramipril, § $p<0.05$ vs *db/db* untreated

mass normalised to TL was reduced in both coenzyme Q₁₀-treated and ramipril-treated *db/db* mice compared with untreated *db/db* controls (Table 2).

Untreated *db/db* mice exhibited significantly hypertrophied cardiomyocytes on H&E-stained sections (Fig. 3a and b), accompanied by elevated gene expression of the hypertrophic marker *Myh7* (Fig. 3c) compared with untreated *db/+* mice. Although coenzyme Q₁₀-treated *db/db* mice also displayed increased cardiomyocyte size and *Myh7* expression compared with *db/+* mice, these parameters were significantly lower than in untreated *db/db* mice. Ramipril-treated *db/db* mice were similarly protected from diabetes-induced increases in cardiomyocyte width and *Myh7* expression.

The cell survival kinase Akt, a downstream target of the IGF1 receptor–phosphoinositide-3 kinase p110 α isoform (IGF1R-PI3K[p110 α]) pathway, is an important mediator of physiological heart growth [23]. In the present study, Akt phosphorylation showed a strong trend to decrease in untreated diabetic mice vs. untreated control mice ($p=0.05$; Fig. 3d). Both coenzyme Q₁₀ and ramipril restored Akt phosphorylation back to levels observed in the non-diabetic mice (Fig. 3d).

Coenzyme Q₁₀ treatment prevents diabetes-induced cardiomyocyte death Diabetes induced a 3.9 ± 0.4 -fold increase in the proportion of apoptotic cells in untreated *db/db* mice compared with *db/+* mice (Fig. 4). Both coenzyme Q₁₀- and ramipril-treated *db/db* mice also exhibited a greater number

Fig. 2 Cardiac fibrosis is reduced in coenzyme Q₁₀-treated mice. **a** Representative Sirius Red-stained LV sections. Collagen appears red; magnification $\times 200$; scale bar 40 μm . **b** Pooled data on quantification of collagen area per visual field. $n=7-12/\text{group}$. *** $p<0.001$ vs *db/+* untreated, § $p<0.05$ vs *db/db* untreated. White bars, *db/+*; black bars, *db/db*

of apoptotic cells compared with their non-diabetic controls. Apoptosis was significantly reduced in both coenzyme Q₁₀-treated *db/db* mice (1.8 ± 0.2 -fold that detected in untreated *db/+* mice) and ramipril-treated *db/db* mice (2.3 ± 0.5 -fold untreated *db/+* mice).

Coenzyme Q₁₀ attenuates oxidative stress in *db/db* mice Plasma concentrations of MDA, a marker of systemic lipid peroxidation, were significantly upregulated in both untreated and ramipril-treated *db/db* mice compared with *db/+* controls (Fig. 5a). Importantly, MDA concentrations in coenzyme Q₁₀-treated *db/db* mice were markedly reduced compared with their untreated counterparts, such that they were no different from the values in coenzyme Q₁₀-treated

Fig. 3 Coenzyme Q₁₀ limits diabetes-induced cardiomyocyte hypertrophy. **a** Representative sections of the H&E stained LV sections; magnification $\times 400$; scale bar 20 μm . The arrow indicates a representative cardiomyocyte width measurement. **b** Pooled data on cardiomyocyte width ($n=10\text{--}11/\text{group}$). **c** Gene expression of hypertrophic marker *Myh7* analysed using real-time PCR and normalised against 18S expression. $n=5\text{--}10/\text{group}$. **d** Quantitative analysis of Akt phosphorylation (phospho-Akt) normalised to total Akt with representative western blot analysis in untreated, Coenzyme Q₁₀- and ramipril-treated hearts. Mean values for *db/+* untreated were normalised to 1. $n=6\text{--}8/\text{group}$. * $p<0.05$ vs *db/+* untreated, *** $p<0.001$ vs *db/+* untreated, † $p<0.05$ vs *db/+* Coenzyme Q₁₀, ‡ $p<0.05$ vs *db/+* ramipril, § $p<0.05$ vs *db/db* untreated. ¶ $p=0.05$. White bars, *db/+*; black bars, *db/db*. CoQ, Coenzyme Q₁₀

db/+ mice. On lucigenin chemiluminescence, untreated *db/db* mice exhibited greater superoxide generation in the myocardium compared with *db/+* controls (Fig. 5b). Coenzyme Q₁₀ showed a strong tendency to reduce superoxide generation ($p=0.05$), whereas ramipril significantly lowered superoxide production in diabetic animals.

Coenzyme Q₁₀ reduces SBP Diabetic *db/db* mice exhibited a significantly higher SBP than non-diabetic *db/+* mice, regardless of treatment group (Fig. 6). Both coenzyme Q₁₀ and ramipril treatment resulted in a modest reduction of SBP (<10 mmHg), which remained significantly elevated compared with untreated diabetic mice. Neither coenzyme Q₁₀ nor ramipril treatment affected diastolic blood pressure (DBP) in the *db/db* mice, or either SBP or DBP in non-diabetic mice (results not shown).

Discussion

The pathophysiology of diabetic cardiomyopathy is multifactorial; cardiomyocyte hypertrophy, interstitial fibrosis and cardiomyocyte death further exacerbate diastolic dysfunction [24]. Hyperglycaemia-induced ROS generation has

been implicated as a key stimulator of these cardiac impairments [25, 26]. Consequently, reducing oxidative stress, in particular excess cardiac superoxide generation, should be favourable in the management of diabetic cardiomyopathy. The major finding of the current study is that the antioxidant coenzyme Q₁₀ protected the heart in the *db/db* mouse model of type 2 diabetes from the development of adverse structural and functional changes, with efficacy comparable to the ACE-I ramipril.

LV diastolic dysfunction is often the earliest hallmark of diabetic cardiomyopathy, preceding the onset of systolic dysfunction [4]. In the current study, diabetes-induced diastolic dysfunction was evident on Doppler echocardiography as prolonged deceleration time, with a similar trend for E:A, indicative of impaired LV relaxation. Diabetes also significantly elevated LVEDP. Importantly, treatment with either coenzyme Q₁₀ or ramipril significantly reduced diastolic dysfunction in terms of all three variables. Previously, Chew and colleagues failed to observe significant effects of coenzyme Q₁₀ on LV diastolic dysfunction in diabetic patients, although a trend towards improved E:A was evident [27]. Importantly, differences in patient characteristics at baseline as well as concomitant medication (ACE-I, statins, angiotensin receptor blockers) were likely to have masked

Fig. 4 Coenzyme Q₁₀ prevents diabetes-induced cardiomyocyte apoptosis. **a** Representative LV sections stained using the CardioTACS In Situ Apoptosis Detection Kit. Positively stained apoptotic cells appear dark blue (indicated by arrows); magnification $\times 200$; scale bar 20 μ m. **b** Quantification of positively stained apoptotic cells/negatively stained non-apoptotic cells, expressed as fold levels detected in untreated *db/+* mouse heart $n=3$ /group. * $p<0.05$ vs *db/+* untreated, † $p<0.05$ vs *db/+* coenzyme Q₁₀, § $p<0.05$ vs *db/db* untreated. White bars, *db/+*; black bars, *db/db*. AU, arbitrary units

coenzyme Q₁₀ cardioprotection [27]. The absence of systolic dysfunction in *db/db* mice is consistent with previous reports in both experimental and clinical settings [19, 28] in which systolic dysfunction manifested later in the disease.

In the present study, cardiac collagen deposition was significantly increased in untreated diabetic mice, consistent with previous findings [29]. Diastolic dysfunction may thus be a secondary consequence of this increased myocardial stiffness. Coenzyme Q₁₀ and ramipril treatment significantly prevented this upregulation of collagen deposition. We also present evidence that type 2 diabetes-induced downregulation of *SERCA2a*, a key modulator

Fig. 5 Coenzyme Q₁₀ reduces oxidative stress in *db/db* mice. **a** Coenzyme Q₁₀, but not ramipril, significantly reduced plasma concentrations of MDA, a marker of lipid peroxidation ($n=6-8$ /group). **b** Ramipril markedly reduced the cardiac generation of superoxide; similar trends were observed with coenzyme Q₁₀ treatment ($n=8-14$ /group). *** $p<0.001$ vs *db/+* untreated, ‡ $p<0.05$ vs *db/+* ramipril, § $p<0.05$ vs *db/db* untreated. † $p=0.05$. White bars, *db/+*; black bars, *db/db*. RLU, relative light unit

of cardiomyocyte contractile function, is significantly ameliorated by coenzyme Q₁₀, suggesting a further role for coenzyme Q₁₀ in protecting the heart against contractile dysfunction, possibly through improved cardiac bioenergetics [30, 31].

Pathological LV hypertrophy is a common structural hallmark of the diabetic heart, and is a strong predictor of myocardial infarction, heart failure and stroke [32]. Our findings show that HW:TL, cardiomyocyte width and *Myh7* expression, all markers of hypertrophy, were upregulated in the hearts of untreated *db/db* mice. Although coenzyme Q₁₀ did not significantly reduce HW:TL in *db/db* diabetic mice, it significantly reduced LV mass (on echocardiography), cardiomyocyte width and *Myh7* expression compared with untreated diabetic mice. Ramipril elicited comparable reductions in LV mass, cardiomyocyte width and *Myh7* expression, as well as decreasing HW:TL.

Fig. 6 SBP is decreased in coenzyme Q₁₀-treated diabetic mice. Both coenzyme Q₁₀ and ramipril treatment marginally reduced SBP in hypertensive diabetic animals. $n=7-11$ /group. *** $p<0.001$ vs *db/+* untreated, † $p<0.05$ vs *db/+* coenzyme Q₁₀, ‡ $p<0.05$ vs *db/+* ramipril, § $p<0.05$ vs *db/db* untreated. White bars, *db/+*; black bars, *db/db*

Coenzyme Q₁₀ treatment has also been shown to elicit beneficial antihypertrophic effects in the setting of hypertrophic cardiomyopathy [33]. The net increase in HW:TL in diabetic mice occurred in the face of increased LV apoptosis, consistent with previous studies [9, 34]. Both hyperglycaemia and oxidative stress can induce apoptosis, further impairing myocardial structure and function, which is implicated in the transition from compensated to decompensated hypertrophy in the diabetic heart [34, 35]. The ability of coenzyme Q₁₀ to reduce diabetes-induced cell death may underlie its effectiveness in reducing myocardial dysfunction and structural remodelling.

Elevated oxidative stress plays a prominent role in the progression of diabetic cardiac complications [8]. Coenzyme Q₁₀ levels are reduced in patients with heart disease and/or diabetes [36], perhaps due to fewer functional mitochondria. In the present study, replenishment of coenzyme Q₁₀ reduced both systemic lipid peroxidation and ventricular superoxide production, in accordance with its ability to reduce oxidative stress and scavenge free radicals in its reduced state [37]. However, it still remains to be determined whether exogenously administered coenzyme Q₁₀ is taken up by mitochondria and reincorporated into the electron transport chain, in addition to increasing circulating coenzyme Q₁₀ [27, 38].

Coenzyme Q₁₀ is detected in membranes other than mitochondria, suggesting that its antioxidant actions are widespread and of physiological importance [30]. Interestingly in the current study, ramipril failed to reduce systemic lipid peroxidation, but it effectively lowered cardiac superoxide production, as previously described [39, 40]. The reduced generation of ROS (and hence restored redox balance) achieved by coenzyme Q₁₀ and ramipril treatment in the present study is likely to be a key mechanism by which they limited myocardial fibrosis and cardiomyocyte hypertrophy in this model of type 2 diabetes, as has been implicated in other settings of cardiac fibrosis [25, 41] and LV hypertrophy [22, 42, 43].

The downstream molecular signalling pathways implicated in ROS-induced cardiac remodelling have not been fully resolved. We demonstrated that cardiac Akt phosphorylation, a downstream target of IGF1R-PI3K(p110 α), was reduced in type 2 diabetes, but was restored with coenzyme Q₁₀ and ramipril treatment. The IGF1R-PI3K(p110 α)-Akt pathway induces a physiological hypertrophic response associated with cardioprotection [19, 23, 44], in which cardiac structure and function are preserved. Based on our observations, we postulate that increased ROS depresses protective IGF1R-PI3K(p110 α)-Akt signalling. The preservation of Akt activity in the coenzyme Q₁₀-treated diabetic myocardium may thereby confer protection against pathological cardiac hypertrophy. This relationship between LV ROS generation and IGF1R-PI3K(p110 α)-Akt signalling warrants further investigation.

Hypertension is a frequent cardiovascular complication in the diabetic population, which further limits patient prognosis [45]. In the current study, SBP was significantly elevated in diabetic mice (in line with previous studies [46]), which was evident in all treatment groups. Although this was modestly reduced by both coenzyme Q₁₀ and ramipril, this reduction was not strongly correlated with cardiomyocyte width reduction; i.e. the effect of coenzyme Q₁₀ and ramipril on reducing cardiomyocyte size was much greater than would be expected based on the modest lowering of SBP. The benefits of coenzyme Q₁₀ on diabetic cardiomyopathy are thus likely to be largely independent of reduced afterload. The mechanism underlying the antihypertensive effect of coenzyme Q₁₀ is inadequately understood, but a reduction in peripheral vascular resistance has been suggested [38, 47, 48]. The antihypertensive actions of ramipril are well known [39, 49].

Limitations of the study Despite the extensive use of *db/db* mice in the study of various diabetes-related pathologies, the *db/db* mouse model is not a perfect model of the human disease. A defect in the leptin receptor subjects *db/db* mice to severe lipotoxicity, more excessive than is observed in diabetes in humans. However, *db/db* mice display a cardiac pathological phenotype that closely mirrors human diabetic cardiomyopathy, including increased cardiac size, depressed cardiac function and efficiency, and impaired Ca²⁺ handling [50]. Despite its limitations, the *db/db* mouse model is considered one of the better animal models for studying the mechanisms underlying human diabetic heart disease and the identification of potential cardiomyopathy-reducing therapeutic agents [50].

Another limitation of our study was the route of coenzyme Q₁₀ administration. The dosage of coenzyme Q₁₀ in drinking water was designed in accordance with average daily water intake of *db/+* and *db/db* mice, and small differences in total dose between animals probably occurred. We acknowledge that oral gavage is a more accurate rate of drug delivery, but this is not well-tolerated by *db/+* or *db/db* mice.

Conclusions

In summary, this study demonstrates the benefit of treating diabetic cardiomyopathy with exogenous antioxidants. Coenzyme Q₁₀ attenuated diabetes-induced diastolic dysfunction, cardiomyocyte hypertrophy, cardiac fibrosis and cell death. The cardioprotective effect of coenzyme Q₁₀ may be mediated through its ability to inhibit systemic oxidative stress, improve cardiac bioenergetics and preserve Akt and SERCA2a regulation in the diabetic context. To our knowledge, our study is the first to establish the ability of

coenzyme Q₁₀ to prevent diabetic cardiomyopathy, and to compare the efficacy of coenzyme Q₁₀ with that of an ACE-I. Considering that ROS-mediated cardiac complications are a major contributor to morbidity and death in the diabetic population, coenzyme Q₁₀ may be a clinically valuable adjunct therapy to standard care for preserving cardiac function in diabetic patients.

Acknowledgements The authors thank N. Jennings for her help with echocardiography, K. Gilbert for her help with animal monitoring and M. A. Arnstein for helpful advice on histological analysis (all from the Baker IDI Heart and Diabetes Institute). Parts of this study were presented in abstract form at the 2010 Scientific Sessions of the American Heart Association, Chicago, IL, USA, November 2010.

Funding This work was supported by the Diabetes Australia Research Trust and the National Health and Medical Research Council of Australia (NHMRC project ID526638 to RHR, JRM and KAJ-D), and supported in part by the Victorian Government's Operational Infrastructure Support Program. KH is supported by an Australian Postgraduate Award. JRM is supported by an Australian Research Council Future Fellowship (FT0001657) and holds an Honorary NHMRC Research Fellowship (586604). X-JD holds an NHMRC Senior Research Fellowship (ID317808). RHR is the recipient of an NHMRC Senior Research Fellowship (ID472673).

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement KH, JRM and RHR contributed to the design of the study, data acquisition, analysis and interpretation of data and drafting of the manuscript. HK, JMF and JEL contributed to data acquisition and interpretation, and manuscript revision. XJD and KJD contributed to data interpretation and manuscript revision. All authors have approved the final version of the manuscript.

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