

R E V I E W

# Diabetic cardiomyopathy

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## A B S T R A C T

Diabetic cardiomyopathy is a distinct primary disease process, independent of coronary artery disease, which leads to heart failure in diabetic patients. Epidemiological and clinical trial data have confirmed the greater incidence and prevalence of heart failure in diabetes. Novel echocardiographic and MR (magnetic resonance) techniques have enabled a more accurate means of phenotyping diabetic cardiomyopathy. Experimental models of diabetes have provided a range of novel molecular targets for this condition, but none have been substantiated in humans. Similarly, although ultrastructural pathology of the microvessels and cardiomyocytes is well described in animal models, studies in humans are small and limited to light microscopy. With regard to treatment, recent data with thiazolidinediones has generated much controversy in terms of the cardiac safety of both these and other drugs currently in use and under development. Clinical trials are urgently required to establish the efficacy of currently available agents for heart failure, as well as novel therapies in patients specifically with diabetic cardiomyopathy.

## INTRODUCTION

The link between HF (heart failure) and diabetes is well documented, but the existence of diabetic cardiomyopathy as a distinct clinical entity continues to be the sub-

ject of debate. In 1881, Leyden commented that HF was a “frequent and noteworthy complication of diabetes mellitus” [1] and Mayer stated that “heart disease in diabetes can be traced to an abnormality in metabolism” [2]. In 1972, Rubler et al. [3] coined the term ‘diabetic

**Key words:** diabetic cardiomyopathy, heart failure, hyperglycaemia, Type 2 diabetes.

**Abbreviations:** ACE, angiotensin-converting enzyme; ACEI, ACE inhibitor; AGE, advanced glycosylation end-product; AngII, angiotensin II; AT<sub>1</sub>, AngII type 1 receptor; ARB, AT<sub>1</sub> receptor blocker; BK, bradykinin; BNP, brain natriuretic peptide; BP, blood pressure; B1R, B1-receptor; B2R, B2-receptor; CHARM, Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity; CHS, Cardiovascular Health Study; CTGF, connective tissue growth factor; DAG, diacylglycerol; DGK, DAG kinase; DPP-4, dipeptidyl peptidase-4; EF, ejection fraction; ERK, extracellular-signal-regulated kinase; ET, endothelin; FA, fatty acid; GLP-1, glucagon-like peptide-1; GLUT4, glucose transporter 4; HbA<sub>1c</sub>, glycated haemoglobin; HF, heart failure; HFNEF, HF with a normal EF; HIF, hypoxia-inducible factor; KLK, kallikrein; hKLK, human KLK; KKS, KLK–kinin system; LV, left ventricular; LVED, left ventricular end-diastole; LVH, LV hypertrophy; LVM, LV mass; LVMI, LVM index; MESA, Multi-Ethnic Study of Atherosclerosis; MHC, myosin heavy chain; MI, myocardial infarction; MR, magnetic resonance; MRI, MR imaging; NCX, Na<sup>2+</sup>/Ca<sup>2+</sup> exchange; NEFA, non-esterified FA; NF-κB, nuclear factor κB; NYHA, New York Heart Association; PARP, poly(ADP-ribose) polymerase; PKC, protein kinase C; PMCA, plasma-membrane Ca<sup>2+</sup>-ATPase; QOL, quality-of-life; RAS, renin–angiotensin system; RAAS, renin–angiotensin–aldosterone system; RAGE, receptor for AGEs; ROS, reactive oxygen species; RR, relative risk; RV, right ventricular; RyR, ryanodine receptor; SERCA, sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup>-ATPase; SHS, Strong Heart Study; SOLVD, Studies of Left Ventricular Dysfunction; SR, sarcoplasmic reticulum; STZ, streptozotocin; TDI, tissue Doppler imaging; TGF-β1, transforming growth factor-β1; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling; TZD, thiazolidinedione; UKPDS, UK Prospective Diabetes Study; VEGF, vascular endothelial growth factor;  $\dot{V}O_{2max}$ , maximal oxygen consumption.

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cardiomyopathy' after performing post mortem studies in diabetic patients with cardiac failure, having excluded alcohol, hypertension, and coronary and structural heart disease as possible aetiologies. Diabetic cardiomyopathy has since been the subject of much research and controversy, with evolving debate on the best management strategy for this entity. By definition, diabetic cardiomyopathy is a distinct primary disease process which develops secondary to a metabolic insult, resulting in structural and functional abnormalities of the myocardium leading to HF. We have comprehensively updated our previous review [4], taking into account the most recent developments in basic and translational research, together with an overview of the latest diagnostic and therapeutic approaches to diabetic cardiomyopathy.

## EPIDEMIOLOGY

The prevalence of HF in the general population ranges from 1 to 4%, but in diabetic patients it is 12% [5], rising to 22% in those over the age of 64 years [6]. Up to a third of all patients admitted to hospital with HF have diabetes [7]. Furthermore, diabetes has a prevalence of 30% in patients with cardiac failure [8] and may be up to four times as prevalent in patients with newly diagnosed HF [9]. Diabetes is also a powerful predictor of cardiovascular morbidity and mortality, and is an independent risk factor for death in patients with established HF [10]. Diabetic patients are also more likely than non-diabetic patients to develop HF following MI (myocardial infarction), despite comparable infarct sizes [11]. The Framingham Heart Study reported a 2.4-fold increase in the incidence of HF in diabetic men and a 5.1-fold increase in diabetic women, when compared with age-matched controls [12]. This association was independent of age, hypertension, dyslipidaemia, obesity and coronary heart disease. Other large population-based studies have yielded similar results [13]. The CHS (Cardiovascular Health Study) of patients aged over 65 years showed diabetes to be associated with an increase in incident HF [14] and the SHS (Strong Heart Study) demonstrated associations between diabetes and higher LVM [LV (left ventricular) mass] and wall thickness, increased arterial stiffness and systolic dysfunction, compared with matched controls [15]. More recently, the MESA (Multi-Ethnic Study of Atherosclerosis) study used cardiac MR (magnetic resonance) to report inter-racial differences in LVM, LV volumes and LV function among diabetic patients [16].

Cross-sectional studies have consistently reported a high prevalence of HF in diabetic populations [6]. Alarming, one study observed 12% of Type 2 diabetic patients with HF at entry, with an annual incidence of 3.3% [17]. In a similar study over 43 months, the incidence of HF was vastly higher in diabetic (39%)

compared with non-diabetic (23%) patients, with an RR (relative risk) of 1.3 for developing HF [18]. The UKPDS (UK Prospective Diabetes Study) found an increased prevalence of HF in Type 2 diabetic patients, which correlated with higher HbA<sub>1c</sub> (glycated haemoglobin) levels [19]. Indeed, for every 1% increase in HbA<sub>1c</sub>, there is an 8% increased risk of developing HF [20]. A recently published study has demonstrated an increased risk of HF in diabetic patients with retinopathy, supporting the concept of a microvascular aetiology in diabetic heart disease [21]. Sub-group analysis of the MESA cohort also revealed an association between retinal arteriolar narrowing and LV remodelling, lending weight to this argument [22].

It is not surprising, therefore, that diabetic patients are over-represented in large HF trial populations; such as SOLVD (Studies of Left Ventricular Dysfunction) [23] (diabetic patients represent 26%), ATLAS (Assessment of Treatment with Lisinopril and Survival) [24] (diabetic patients represent 19%), V-HeFT (Vasodilator-Heart Failure Trial) [25] (diabetic patients represent 20%) and RESOLVD (Randomized Evaluation of Strategies for Left Ventricular Dysfunction) [26] (diabetic patients represent 27%). In addition to overt clinical diabetes, insulin resistance and the metabolic syndrome are also independent predictors of HF [27,28]. Inflammatory markers such as CRP (C-reactive protein) and IL (interleukin)-6, as well as microalbuminuria, which are frequently elevated in individuals with the metabolic syndrome, are independently associated with incident HF [29]. This suggests that cardiomyopathy may occur in 'pre-diabetic' individuals. In a retrospective study of a cohort of patients with a primary diagnosis of HF, metabolic syndrome was present in 68% of patients, but, interestingly, mortality was lower in those with (44%) compared with those without (58%) the metabolic syndrome [30].

## RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF HF IN DIABETES

### Hyperglycaemia

In the UKPDS, although poor glycaemic control was associated with an increased risk of HF [19], intensive glycaemic control did not reduce the risk of incident HF. A more recent study of Type 2 diabetic patients has, however, demonstrated an improvement in long-axis function (systolic strain rate) and reductions in LVM, which were associated with improvements in HbA<sub>1c</sub> and fructosamine levels [31]. These changes were independent of any BP (blood pressure)-lowering effects. In addition, there was an improvement in diastolic function during the first 3 months of follow up, which returned to baseline at 12 months. Another study by Marwick and co-workers [32] has reported a similar association between lower

HbA<sub>1c</sub> and improved systolic strain. In a 10 year follow-up of the UKPDS, despite an early loss of glycaemic difference between groups receiving intensive and conventional treatment, an emergent risk reduction for MI and death from any cause was observed following intensive glucose-lowering [33], although no data was reported for HF. However, conflicting data has been generated from two recent large randomized intervention trials. The ADVANCE (Action in Diabetes and Vascular Disease) trial compared intensive with standard glycaemic control and showed a non-significant reduction in incident HF, but an increased all-cause mortality at 3.5 years [34]. Increased mortality in the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial led to the discontinuation of the intensive glycaemic control arm of the study [35].

### Hypertension

Hypertension is independently associated with LVH (LV hypertrophy), diastolic dysfunction, HF and cardiovascular risk [4]. In the UKPDS, lower BP, achieved by either a  $\beta$ -blocker or ACEI [ACE (angiotensin-converting enzyme) inhibitor], was associated with a reduced risk of incident HF compared with less intensive BP control. However, again it is interesting to note that both during and in the recent 10 year follow-up of the UKPDS, BP reduction did not demonstrate the 'legacy effect', i.e. there was no risk reduction during or after the trial for MI or death from any cause [36].

## STRUCTURAL FEATURES OF DIABETIC CARDIOMYOPATHY AND THEIR FUNCTIONAL RELEVANCE

### LVH

LVH is a powerful predictor of cardiovascular risk. The Framingham Heart Study demonstrated an increased relative risk of developing cardiovascular disease for each 50 g/m<sup>2</sup> increment in LVMI (LVM index; RR 1.49 compared with 1.57, males compared with females) [37]. Furthermore, LVH predicts prognosis in high-risk patient groups, such as those with coronary heart disease [38], HF [39], diabetes [40], renal failure [41], hypertension [42], obesity and previous MI [43]. Pharmacological regression of LVH is associated with a reduction in cardiovascular risk [44]. The RENAAL [Reduction of Endpoints in NIDDM (non-insulin-dependent diabetes mellitus) with the Angiotensin II Antagonist Losartan] study in patients with diabetic nephropathy, found that LVH was a significant risk factor for the primary endpoints of end-stage renal failure, death or cardiovascular events [45]. Several studies have since reported an association between diabetes and LVH [46]. The SHS reported increased LVM and LV wall thickness in both diabetic men and women, and these differences remained

statistically significant in multivariate analyses after adjusting for confounding factors [47]. Similar findings were reported in the CHS [48] and MESA [49], as detailed above. Interestingly, a sub-study of the MESA cohort reported an independent association between retinal arteriolar narrowing and LV remodelling, which was stronger in diabetic patients, suggesting microvascular involvement [22]. The Framingham Heart Study also reported increased LVM across all categories of glucose dysmetabolism. A recent study of Japanese Type 2 diabetic patients reported an association between insulin resistance, arterial stiffness and LVMI [using cardiac MRI (MR imaging)] [47], and this has been supported by a large population-based study in Sweden, which demonstrated associations between metabolic syndrome, insulin resistance and increased LVM and LV wall thickness [48].

### Diastolic dysfunction

Diastolic function of the left ventricle is determined by its passive elastic properties, coupled with the process of active relaxation. Diastolic dysfunction is characterized by impairment of relaxation and passive filling of the left ventricle [49], and diastolic HF is said to exist when diastolic dysfunction is associated with an elevated end diastolic pressure, clinical features of HF and a normal EF (ejection fraction). This may be better termed HFNEF (HF with a normal EF), since there is emerging evidence of subtle abnormalities in regional and long-axis systolic function in some of these patients.

Functional abnormalities occur as a result of structural remodelling (concentric LVH) and result in normal or near-normal end diastolic volume, elevated LVM to volume and elevated wall thickness to chamber radius relationships respectively. Indeed, the development of diastolic dysfunction has been associated with only modest increases in LVM [47]. In diabetic cardiomyopathy with reduced LVEF, myocardial collagen deposition and AGEs (advanced glycosylation end-products) are the primary pathological processes responsible for reduced elasticity of the myocardium, whereas increased cardiomyocyte resting tension may be the predominant cause in those cases with preserved LVEF [50]. LVH and geometric remodelling on the other hand, cause an increase in passive stiffness and impaired relaxation. Consequently, the LV pressure–volume curve is shifted upward and leftward, chamber compliance is reduced, diastolic filling is altered with an elevation of the end diastolic pressure, and HF ensues.

Diastolic dysfunction is a common finding in otherwise healthy and asymptomatic diabetic patients and is thought to be the earliest detectable functional abnormality in diabetic cardiomyopathy [51]. In a study of normotensive, asymptomatic Type 2 diabetic patients with good glycaemic control, 47% were found to have diastolic dysfunction [52]. Other studies using more

sensitive diagnostic methods have reported that as many as 75% of diabetic patients demonstrate abnormalities of diastolic function [53].

### Systolic dysfunction

Advances in TDI (tissue Doppler imaging) have allowed for measurement of sensitive indices of regional and long-axis function of both the left and right ventricles [54]. Indeed, long-axis function is emerging as a superior prognostic indicator to EF, especially in patients with HFNEF [55]. Using these sensitive methods, several studies have demonstrated subtle abnormalities in systolic function in patients with a diagnosis of diastolic dysfunction [56–58]. This has led some to question whether diastolic dysfunction exists in isolation at all [59], whereas others have questioned the relevance of these subtle systolic abnormalities in the context of diastolic dysfunction [60]. The longitudinal fibres responsible for long-axis contraction lie in the sub-endocardium and are particularly susceptible to the effects of fibrosis, ischaemia or hypertrophy. In diabetic patients with HFNEF, long-axis systolic dysfunction is associated with a compensatory increase in radial thickening and mass, thus preserving LVEF [61]. The preservation of EF is directly related to the presence of LVH and the effect of increased muscle mass. Patients with HFNEF have significantly higher LVMI, lower LVED (left ventricular end-diastole) volume index, higher LVMI/LVED volume index ratio than patients with dilated left ventricles and a low EF. Although most studies to date have concentrated on LV function, the importance of RV (right ventricular) function should not be overlooked; RVEF has previously been shown to be an independent predictor of poor outcome [62]. Furthermore, RV diastolic dysfunction has been reported in asymptomatic patients with Type 1 diabetes [63].

### DIAGNOSTIC METHODS

Although no single diagnostic test for diabetic cardiomyopathy exists, using different imaging modalities it is possible to detect the phenotypic cardiac features of this condition. Currently used diagnostic methods in clinical practice include echocardiography, cardiac MR and cardiac biomarkers such as NT-BNP [N-terminal pro-BNP (brain natriuretic peptide)].

#### Echocardiography

Echocardiography is an excellent non-invasive and practical imaging tool for defining cardiac structure and function and allows 'real-time' visualization of the cardiac cycle. Quantitative and qualitative assessment of the heart can be made with regard to LV geometry, regional wall motion, and systolic and diastolic function, in addition to valvular anatomy and function. Two-dimensional echo-

cardiography has traditionally been the method of choice in detecting and quantifying LVH, and has been validated in the research and clinical setting. The main limitation of two-dimensional echocardiography is for patients with major distortions of LV geometry. As a result, most existing data from studies of LVH and LVM have been derived using standard two-dimensional and M-mode echocardiography. Although considered to be the 'gold standard' for assessing LV diastolic function, cardiac catheterization is not essential to diagnose diastolic dysfunction [63]. Pulsed-wave Doppler echocardiography is therefore the most practical and commonly used method for current assessment of diastolic function. A detailed and comprehensive diastolic study is vital in diabetic patients and should include the measurement of transmitral and pulmonary venous flow/velocities, as well as left atrial volume [64]. LVEF is the most commonly used index of LV systolic function and is derived echocardiographically. Despite being well established in clinical practice, it is important to be aware that it can be influenced by alterations in preload and afterload.

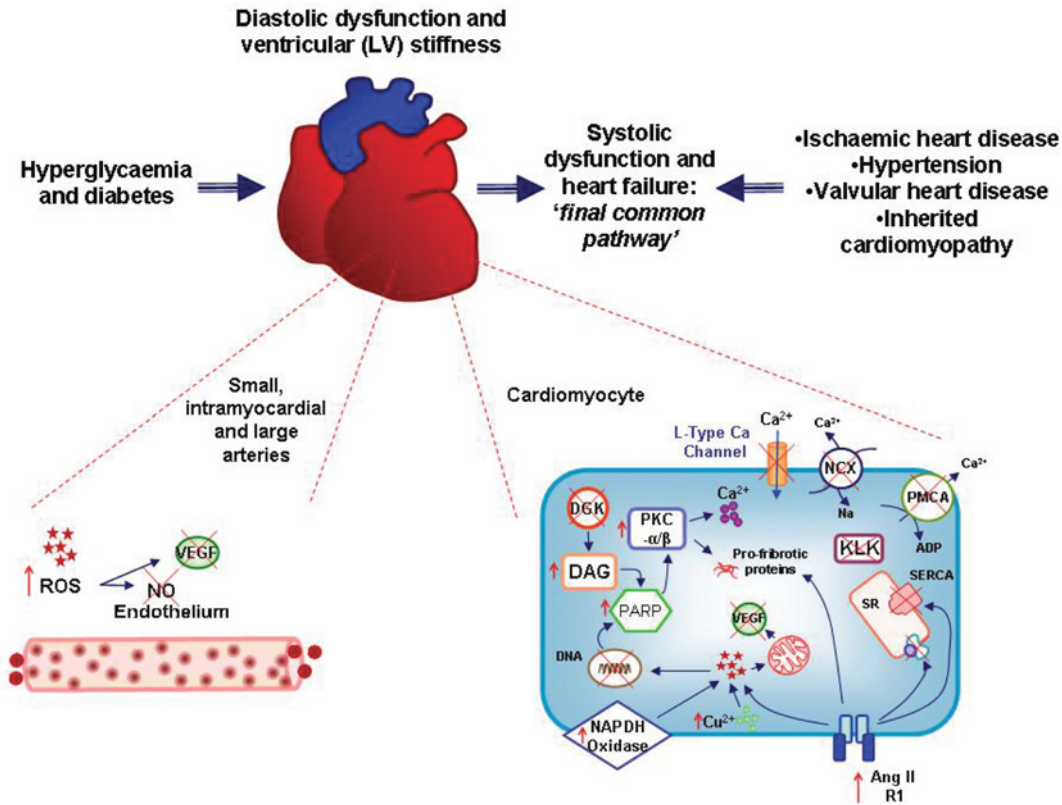
LVEF is not as sensitive for assessing contractile function in patients with HFNEF and TDI echocardiography is more capable of detecting subtle regional abnormalities in LV function. TDI allows non-invasive assessment of myocardial strain and has been shown to identify global and regional abnormalities in myocardial properties, with a high level of temporal resolution. TDI differs from conventional Doppler in that it utilizes a filter which eliminates high velocity and low amplitude signals reflected from blood cells, thereby allowing low velocity, high amplitude tissue signals to be analysed. Myocardial strain and strain rate are dimensionless indices of changes in length that reflect tissue deformation [65]. These parameters have been extensively validated in a range of different disease states, including acute ischaemic syndromes [66], and have been found to be indicative of preclinical myocardial disease in patients with Type 2 diabetes [57].

#### Cardiac MRI

Cardiac MRI, owing to its superior accuracy and reproducibility, is the 'gold standard' for measuring LVM [67]. Currently however, its use is mainly limited to research, due to costs, time constraints and the expertise required for assessment.

#### Cardiac biomarkers

BNP is a cardiac hormone secreted in response to ventricular volume and pressure overload. Although it is both sensitive and specific for congestive HF, it cannot reliably distinguish between systolic and diastolic HF, which limits its diagnostic use in diabetic cardiomyopathy [56,68].



**Figure 1** Summary of interactions between the myocardial and vascular changes present in diabetic hearts and their contribution to diabetic cardiomyopathy and heart failure

Ang II R1, AT<sub>1</sub>; IGF-I, insulin-like growth factor-I.

## MOLECULAR BASIS

Several putative factors have been considered important in the genesis of the functional and structural alterations which lead to the development and progression of diabetic cardiomyopathy (Figure 1). The molecular basis of this condition has been established primarily from studies in experimental models of diabetic cardiomyopathy [69], with few studies carried out in humans [4].

### Hyperglycaemia

Several studies, most notably the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study, have shown that hyperglycaemia is indeed a mediator of cardiovascular risk in Type 1 diabetes and that intensive diabetic therapy reduces cardiac outcomes via an impact on accelerated atherosclerosis, cardiac autonomic neuropathy and possibly cardiomyopathy [70]. In a population-based cohort study of 746 subjects stratified into normal and abnormal glucose tolerance and overt Type 2 diabetes, there was an independent increase in risk of developing systolic (2-fold) and diastolic (2.4-fold) dysfunction in patients with Type 2 diabetes, with diastolic dysfunction apparent even in those with impaired glucose tolerance [71].

Hyperglycaemia mediates its damaging effects through a series of secondary transducers which will be considered.

### ROS (reactive oxygen species) and NO

Oxidative stress occurs when the production of ROS outweighs their degradation by antioxidant defences and the elevation of ROS leads to cellular damage by oxidation, disruption of vascular homeostasis through interference with NO and, most recently, by modulation of detrimental intracellular signalling pathways. ROS have been implicated in all stages of the development of HF, from cardiac hypertrophy to fibrosis, contractile dysfunction and failure [71]. Diabetic hearts exhibit a similar picture to that of failing hearts, with numerous studies reporting increased production of ROS in a variety of animal models of diabetes [71]. Increased ROS causes cardiac dysfunction by direct damage to proteins and DNA {inducing PARP [poly(ADP-ribose) polymerase] which will be considered later}, as well as by promoting apoptosis. Furthermore, overexpression [72] or pharmacological administration [73] of the antioxidant metallothionein in rodent models of diabetes has been shown to ameliorate diabetic cardiomyopathy. Similar results have been reproduced using other antioxidants in

both Type 1 and Type 2 rodent models of diabetes [74]. NADPH oxidase enzymes are a source of ROS and are involved in redox signalling by acting as catalysts for electron transfer from NADPH to molecular oxygen, resulting in the generation of free radicals [75]. They comprise multisubunit complexes, which range from NOX1 to NOX5 [75]. Through interaction with a variety of transcription factors, redox signalling influences the expression of growth-related genes and in-turn effects contractile function [76]. NADPH oxidase is increased in both failing [77] and diabetic [78] rodent hearts and this relationship is believed to be causal. In diabetic animals, up-regulation of NADPH oxidase correlates with cardiac hypertrophy and up-regulation of pro-fibrotic genes such as pro-collagen III; changes which can be ameliorated using the antioxidant Tempol [79]. ROS also react directly with NO to form peroxynitrite species, thereby inactivating the vasodilatory effect of NO, which is integral to vascular homeostasis and endothelial function. In combination with other pathology within the endothelium [80], these changes provide an explanation for accelerated endothelial dysfunction seen in prediabetic mice [81]. Interestingly, antioxidants such as vitamin C are capable of restoring endothelial function in patients with HF [82]. Reductions in the release of NO from endothelial cells, as a consequence of ROS, have also been shown to affect ventricular relaxation in LVH [83]. Thus experimental data suggest that regimes targeting reduction of ROS or increasing antioxidant activity potentially represent novel therapeutic modalities for diabetic cardiomyopathy. However, clinical studies to date have been unable to translate these results to the clinic, with several large randomized trials failing to report a benefit of antioxidants on cardiovascular outcome in high-risk individuals [84]. The outcome of such trials in patients with diabetic cardiomyopathy however, has not been investigated.

### PARP

PARP enzymes are overactivated in diabetes [85] as a reparative response to ROS-induced oxidative damage to DNA (see above). PARP inhibits GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which leads to accumulation of glycolytic intermediates, which in-turn activate a series of transducers which inflict tissue damage via AGE formation and PKC (protein kinase C) activation [85]. PARP also promotes cardiac damage by activating NF- $\kappa$ B (nuclear factor  $\kappa$ B) [87] and inducing overexpression of the vasoconstrictor ET (endothelin)-1 and its receptors [88]. Both hyperhexosaemia and hyperglycaemia have been shown to induce oxidative stress and up-regulate extracellular matrix and cardiomyocyte hypertrophy and these changes were effectively abolished in PARP-1 knockout (*Parp*<sup>-/-</sup>) mice and in rats treated with the PARP inhibitor ABA (3-aminobenzamide) [89]. PARP-driven cardiac

hypertrophy and fibrosis appears to be mediated in hearts and cardiomyocytes via up-regulation of the transcriptional co-activator p300 [89]. Thus the widespread damage caused by PARP potentially creates a unique opportunity for blocking multiple sources of cardiac injury and has been shown to inhibit many pathways thought to mediate myocardial damage in diabetes [85,90]. Additionally, PARP inhibition has been shown to reverse diabetic endothelial dysfunction [91]. Again, although the experimental data appear compelling, as yet no PARP inhibitors have been developed for clinical use, due to off-target, and perhaps on-target, drug toxicity.

### PKC

PKC activity is increased in both failing [92] and diabetic [93] hearts, and levels correlate with both ROS [94] and PARP [85]. PKC phosphorylates a number of proteins directly involved in cardiac excitation-contraction coupling and therefore disturbs Ca<sup>2+</sup> handling in cardiomyocytes [95]. Transgenic mice overexpressing the PKC $\beta$ 2 isoform in the myocardium develop cardiac hypertrophy, fibrosis, impairment of LV function and progressive cardiomyopathy, but these changes are reversed using a PKC $\beta$  isoform-selective inhibitor [96]. DAG (diacylglycerol) activates PKC and is phosphorylated by DGK (DAG kinase), converting it into phosphatidic acid and thereby inactivating its function in respect to DAG [97]. It has recently been shown that overexpression of the DGK $\zeta$  isoform in both cultured mouse cardiomyocytes and isolated perfused hearts [98], as well as diabetic mice [99], prevents pathological activation of PKC, attenuating cardiac hypertrophy and fibrosis and improving ventricular function. Conversely, targeted overproduction of the PKC $\beta$ 2 isoform in the myocardium results in LVH, multifocal fibrosis and cardiomyopathy in mice [96]. CTGF (connective tissue growth factor), which contributes to cardiac hypertrophy and fibrosis is overexpressed in concert with PKC $\beta$ 2 in rodent models of diabetic cardiomyopathy [93]. Treatment with antioxidants is capable of normalizing this cardiac hypertrophy and reducing CTGF levels, therefore implicating oxidative stress as a potential mediator of PKC $\beta$ 2-induced myocardial damage [100]. Inhibition of PKC $\alpha$  is also associated with significant improvements in cardiac function in rodent models of HF [101], in conjunction with an improved metabolic gene profile in the myocardium, as well as improved glucose utilization and diastolic function as shown by MR spectroscopy [102].

### AGEs

Hyperglycaemia promotes formation of collagen types I and III in the myocardium, resulting in interstitial fibrosis, which leads to LV diastolic dysfunction; however, the mechanisms of hyperglycaemia-induced collagen production are poorly defined. The increased

formation of AGEs secondary to hyperglycaemia, may alter structural proteins and lead to increased myocardial stiffness. Aminoguanidine (an inhibitor of AGE formation and protein cross-linking) has been shown to ameliorate changes in LV structure and function [103]. Hyperglycaemia induces oxidative stress which increases profibrogenic factors leading to interstitial fibrosis, a key alteration in diabetic cardiomyopathy. Oxidative stress increases expression of AGE and RAGE (receptor for AGEs) leading to an activation of NF- $\kappa$ B which leads to a switch in the gene expression of cardiac MHC (myosin heavy chain) from the  $\alpha$ -MHC isoform to the  $\beta$ -MHC isoform altering myocardial contractility [104]. DHEA (dehydroepiandrosterone) reduces oxidative stress and in turn has been shown to reduce tissue levels of collagen I, collagen IV and fibronectin in STZ (streptozotocin)-rats and restore papillary muscle contractility [105]. Elevated levels of RAGE, NF- $\kappa$ B and TGF- $\beta$ 1 (transforming growth factor- $\beta$ 1) mRNA in myocardial tissue of diabetic rats was reduced significantly after administration of grape seed proanthocyanidin extract and this was associated with a decrease in the number of degenerate mitochondria and the preservation of ultrastructural alterations in LV myocardium [106]. In an *in vitro* study of cardiac fibroblasts exposed to a high-glucose concentration, ERK1/2 (extracellular-signal-regulated kinase 1/2) activation led to enhanced mRNA and protein expression of collagen types I and III, which was ameliorated by treatment with a blocker of ERK phosphorylation [107].

### Fatty acids

Independent of the effects of hyperlipidaemia on coronary artery endothelial function, diabetic hearts have an altered metabolic phenotype, with enhanced FA (fatty acid) utilization. A recent study in *db/db* mice, a monogenic model of Type 2 diabetes with extreme obesity and hyperglycaemia, has demonstrated increased plasma membrane content of FA transporters [FAT/CD36 and FABPpm (membrane associated FA-binding protein)], leading to increased FA uptake and utilization in *db/db* cardiomyocytes [105]. This has been assumed to be driven by a range of mitochondrial mechanisms, but there was no change in CPT-1 (carnitine palmitoyltransferase-1) activity, malonyl CoA and UCP (uncoupling protein)-3 content suggesting that mitochondrial mechanisms do not contribute to elevated rates of FA oxidation in *db/db* hearts [105].

### Dysfunctional calcium homeostasis

Calcium is one of the principal ionic regulators in the heart and is essential for the process of excitation–contraction coupling and therefore integral to normal cardiac function. Thus, during the cardiac action potential, the cell membrane of the cardiomyocyte is depolarized and calcium enters the cell through voltage-dependent L-type

calcium channels in the sarcolemma. Calcium triggers the release of further calcium ions from the SR (sarcoplasmic reticulum) store, through the RyRs (ryanodine receptors), which increase intracellular calcium and facilitate binding of calcium to myofilaments, thereby initiating cardiac contraction. For relaxation to occur, calcium ions must be removed from the cytosol, the majority of which is pumped back into the SR by SERCA (sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase), while the remainder is ejected out of the cell through the sarcolemmal NCX ( $\text{Na}^{2+}/\text{Ca}^{2+}$  exchange), PMCA (plasma-membrane  $\text{Ca}^{2+}$ -ATPase) or mitochondrial calcium uniport [106]. In both Type 1 and Type 2 rodent models of diabetes, altered expression, activity and function of all transporters involved in excitation–contraction coupling, SERCA [107], NCX [108], RyR [109] and PMCA [110], as well as dysfunctional intracellular calcium signalling [111], have been reported. These findings echo calcium mishandling observed in HF [106]. Interestingly, candesartan, an ARB {AT<sub>1</sub> [AngII (angiotensin II) type 1] receptor blocker}, has been shown to restore the contractile deficit in diabetic cardiomyopathy by stabilizing FKBP (FK506-binding protein) 12.6 and restoring calcium release through the RyR [112]. Depressed SERCA activity causes inefficient sequestration of calcium in the SR, resulting in cytosolic calcium overload, impaired relaxation and hence diastolic dysfunction [113]. Overexpression of SERCA has been shown to improve calcium handling [111] and protect against experimental diabetic cardiomyopathy [107]. In a study utilizing myocardial biopsies in seven diabetic patients with diastolic dysfunction, myofilament  $\text{Ca}^{2+}$  responsiveness was found to be reduced [114]. In addition to alterations in calcium homeostasis, there is also reduced expression of mRNA and protein density of key cardiac K<sup>+</sup> channel (Kv2.1, Kv4.2, and Kv4.3) genes in LV myocytes in experimental diabetes. This will contribute to repolarizing K<sup>+</sup> currents and explain the susceptibility to arrhythmia in diabetic cardiomyopathy [115].

### RAAS (renin–angiotensin–aldosterone system)

The involvement of the RAS (renin–angiotensin system) in HF has now begun to be defined at the molecular level in relation to HF and diabetic cardiomyopathy. AngII exerts a direct effect on cardiomyocytes through AT<sub>1</sub> receptors [116]. Both diabetes and hyperglycaemia induce functional abnormalities in ventricular myocytes, which can be prevented by AngII blockade [117]. The mechanistic basis for this dysfunction is not clear; however, direct signalling via the AT<sub>1</sub> receptor results in increased NADPH oxidase activity and elevation of ROS which causes oxidative damage to cardiomyocytes and endothelial cell apoptosis [117]. In diabetes an up-regulation of RAS occurs despite minimal changes in

myocardial loading and increased expression of AngII in diabetic rats has been related to cardiomyocyte hypertrophy and apoptosis. Six weeks of STZ-diabetes in Ren-2 (enhanced tissue renin–angiotensin expression) rats results in impairment of both active and passive phases of diastole with interstitial fibrosis, cardiac myocyte hypertrophy and apoptosis in conjunction with increased TGF- $\beta$  activity [118]. These findings provide a molecular platform for the increased benefits of ACE inhibition in diabetic patients in preventing and reversing diabetic cardiomyopathy.

AngII receptor blockers attenuate metabolic and cellular alterations in experimental diabetes [119], reduce ROS [120], restore SERCA activity and improve intracellular  $\text{Ca}^{2+}$  handling in HF [121]. It is therefore not surprising that angiotensin blockade has been shown to provide cardiovascular protection in diabetic patients [122]. Aldosterone antagonists also reduce cardiovascular mortality in diabetic patients with HF who exhibit elevated serum levels of collagen synthesis markers [123], suggesting that aldosterone antagonists may prevent excessive extracellular matrix turnover. As both AngII and aldosterone induce cardiac fibrosis, through enhanced accumulation of collagen and increased fibroblast proliferation [124], it has been suggested that aldosterone and glucose mediate cardiac fibrosis through stimulation of myofibroblast growth in patients with a dysregulated RAAS, a pathway of cardiac pathology which is intensified by hyperglycaemia [125].

### **HIF (hypoxia-inducible factor)-1 and VEGF (vascular endothelial growth factor)**

An adequate angiogenic response to hypoxia is essential in protecting against myocardial injury during ischaemic events. The hypoxic stimulus is mediated largely by HIF-1, a transcriptional regulator complex which controls the expression of multiple angiogenic growth factors, of which VEGF has received particular attention [126]. The presence of hypoxia and free radicals stabilize and activate HIF-1 $\alpha$ , which would otherwise be rapidly degraded [127]. VEGF has been shown to contribute to the development of collateral vessels [128] and is expressed in increased quantities in cardiac myocytes and arteriolar smooth muscle cells following MI in non-diabetic patients [129]. However, the expression of VEGF protein and mRNA, as well as its receptors, are significantly decreased in the myocardium of both diabetic and insulin-resistant non-diabetic rats [130]. This suggests that, in diabetic patients, the normal molecular processes which regulate angiogenesis may be impaired, although there are no direct studies to date to substantiate this. This may provide an explanation for the increased incidence of post-MI cardiac failure and enhanced mortality in diabetic patients. Interestingly, administration of TZDs (thiazolidinediones) [131] or inhibition of NADPH

oxidase [132] results in an increase in circulating VEGF levels in diabetic patients and animals respectively, with restored post-ischaemic neovascularization in the latter; implicating ROS activity once more in the pathology of diabetic cardiomyopathy. Reductions in VEGF and impaired angiogenic responses have also been linked with increased levels of ET-1 in ventricles from diabetic rodents and ET receptor antagonism increases VEGF signalling, improving cardiac function [133].

### **The KKS [KLK (kallikrein)–kinin system]**

There is now good evidence that the KKS has a profound influence on cardiac and vascular function. Tissue KLK is involved in processing vasoactive kinin peptides and appears to have dynamic roles, with reports of both protective and damaging effects on the cardiovascular system [134,135]. These effects are mediated through two distinct G-protein-coupled receptors; the BK (bradykinin) B1R (B1-receptor) and B2R (B2-receptor). Under basal conditions, BK and Lys-BK (kallidin) are the predominant kinin peptides involved in cardiovascular homeostasis and work principally through activation of the B2R. The B1R has been shown to mediate cardiac inflammation and may therefore contribute to cardiovascular pathology [135].

A range of cardiovascular insults, including oxidative stress, inflammatory cytokines and activation of the RAS have been shown to regulate these receptors [136]. The STZ-induced diabetic rat exhibits up-regulation of both B1R and B2R [137], which may partly be explained by activation of the RAS in diabetes [134]. Paradoxically, KLK levels are reduced in diabetic animals [138] and humans [139], suggesting therefore that kinin receptor up-regulation may represent an attempted compensatory mechanism [135].

The relationship between these cardiovascular insults and levels of KLK/BK receptors is reciprocal. Alterations to B2R expression by use of transgenes in experimental models of diabetes has demonstrated protection against diabetic cardiomyopathy. BK has been shown to improve insulin stimulation of GLUT4 (glucose transporter 4) [140] via a BK B2R-induced NO pathway [141]. Following somatic hKLK (human KLK) gene delivery to STZ-induced diabetic rats, there is increased cardiac GLUT4 translocation, decreased cardiac glycogen synthesis and reduced glycogen accumulation [142], suggesting that the KKS is capable of restoring glucose utilization. Furthermore, hKLK transgenic STZ-induced diabetic rats show a reduced cardiac inflammatory response [143], increased levels of antioxidative enzymes [144], inhibition of collagen accumulation and restoration of SERCA2a-associated calcium disturbances [145]. Functionally, these changes are associated with subsequent improvement in LV filling pressure/stiffness [146].

Thus there is clearly extensive cross-talk between the KKS and the cardiovascular system. Interactive



pathways between the KKS and the RAS, myocardial energy metabolism, oxidative stress, inflammation and calcium handling, are complex and dynamic with respect to the BK receptor involved. Furthermore delineation of these mechanisms may allow manipulation of this system, potentially via blockade of the RAS, utilizing readily available therapy (ACEIs, ARBs and renin inhibitors) to selectively oppose the alterations which contribute to diabetic cardiomyopathy in man.

## **PATHOLOGY**

As a consequence of the molecular changes discussed, characteristic structural alterations develop in the cardiomyocytes and intramyocardial blood vessels of diabetic individuals, contributing to the pathogenesis of diabetic cardiomyopathy.

### **Cardiomyocytes**

Since the cardiomyocyte is the primary functional unit of the myocardium, it has been the focus of studies investigating diabetic cardiomyopathy. Light and electron microscopic ultrastructural changes of the cardiomyocyte have been defined in a range of animal models of diabetes, including the dog [147], monkey [148], rabbit [149], mouse [150] and rat [151–154]. However, widely varying results have been reported with regard to the severity, onset and type of cardiomyocyte pathology. Hence the pathology has ranged from no change [147] to severe myocytolysis and contracture band formation [151]. With regard to onset of alterations, some studies have demonstrated significant changes within 1 week [155], whereas others only detected changes after 6 [153] and 12 weeks [151] of experimental diabetes. Possible explanations for these discrepancies, other than the duration of diabetes, include the severity of hyperglycaemia and the potential direct effect of the diabetogen used to induce diabetes. Thompson [153] induced diabetes in the Sprague–Dawley rat using a single injection of 4% alloxan monohydrate, and by light microscopy demonstrated a progressive increase in intercellular and perivascular matrix after 6 weeks of diabetes. However, this abnormality was not expressed uniformly and there was regional heterogeneity, with areas showing no increase in matrix [153]. More detailed ultrastructural assessment using electron microscopy demonstrated significant ultrastructural alterations in only 15% of the cardiomyocytes, which were characterized by: (i) loss of myofibrils with disorganization of the remaining bundles, (ii) areas of separation at the intercalated disk along the fasciae adherents, and (iii) increased lipid droplets and loss of some elements of the SR and transverse tubules [153]. A longer duration of diabetes leads to the development of more pronounced degenerative changes in larger groups of cardiomyocytes and, at 26 weeks, approx. 60% of

the cells were affected, displaying large areas of free cytoplasm, dispersed proteins, contracture bands and a reduction in the frequency of SR and transverse tubules. However, interestingly, most of the mitochondria appeared normal with no evidence of swelling or lysis and morphology of the cristae remained normal, despite the presence of severe damage and disorganization of the cytoplasm with contracture band formation. This is in contrast with other ultrastructural studies, which have shown swollen and fragmented mitochondria as the most prominent finding in diabetic myocardium [149]. Indeed, mitochondrial swelling has been observed in rats after 7 days of hyperglycaemia [155], as well as in dogs [156]. A number of other studies have reported disruption and loss of mitochondria in more advanced stages of diabetic cardiomyopathy [150–152].

Studies in humans are limited. Fischer et al. [157] undertook detailed ultrastructural studies of tissue plugs from the anterior apical segment of the heart in 145 patients undergoing CABG (coronary artery bypass grafting). Subjects were divided into three groups: ‘overtly diabetic’, ‘chemically diabetic’ (representing those with impaired glucose tolerance) and ‘euglycaemic/non-diabetic’ patients. The study showed cardiomyocyte hypertrophy and interstitial fibrosis in all except two samples. In addition, a spectrum of other myocardial lesions were observed in all three groups of patients, ranging from mitochondrial degeneration and fatty infiltration of the myofibrils to contraction band formation, perivascular and interstitial oedema and myocytolysis. The study thus failed to detect specific alterations in the cardiomyocytes of diabetic patients, as all changes were also present in non-diabetic patients [157]. The one ultrastructural alteration which distinguished diabetic from non-diabetic patients was capillary basement membrane thickening, which will be discussed below. However, it is important to note that the criteria used to group the patients was not precise and overlap between the groups could have resulted from ambiguous terminology, dividing patients into ‘overtly diabetic’ and ‘chemically diabetic’.

With regard to the size of cardiomyocytes, contrasting studies in diabetic patients and animal models demonstrate both cardiomyocyte hypertrophy and atrophy. Cardiomyocyte apoptosis has been demonstrated in the diabetic myocardium of animal models [158], as well as in humans [159]. In STZ-induced diabetic rats [160], TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) detected 3-OH strand breaks resulting from DNA breakdown. The nuclear marker DAPI (4',6-diamidino-2-phenylindole) was used to counterstain DNA and cells defined as apoptotic if the nuclei showed a sharply demarcated morphology and were labelled by the TUNEL assay, were increased 3.3-fold in diabetic rats, although the overall incidence of this abnormality was very low [160]. However, it

**Table 1** Summary of the major structural changes in experimental and human diabetic cardiomyopathy

↑ indicates increase; ↓ indicates decrease; = indicates no difference. BB, BioBreeding; ZDF, Zucker diabetic fatty.

Diabetes type	Model	Cardiac mass	Cardiomyocyte diameter	Cardiomyocyte loss	Mitochondria	SR	Intramyocardial Apoptosis lipid	Fibrosis
Type 1 diabetes	STZ rat	= [151] or ↓ [166,201]	↓ [166,201]	↑ [151]	↑ [151,155,201]	↑ [151,155]	↑ [158] ↑ [151,155]	= [201] or ↑ [166]
	Alloxan rat	= [153]		↑ [153]	= [153]	↑ [153]		↑ [153]
	BB Wistar rat	↓ [152]		↑ [152]	↑ [152]	↑ [152]	↑ [152]	↑ [152]
Type 2 diabetes	KK mice			↑ [163]	↑ [163]	↑ [163]		↑ [163]
	ZDF rat	= but after insulin ↑ [202]	↑ [202]				↑ [203]	↑ [202]
	<i>ob/ob</i> Mice	↑ [150,204]	↑ [204] or ↓ [150]	↑ [150]	↑ [150]		= [204] or ↑ [150]	= [204]
Type 1 and 2 diabetes	Humans	↑ [205,206]	↑ [207,208] or ↓ [209]	= ? [157]	= ? [157]	= ? [157]	↑ [159] = ? [157]	= [157] or ↑ [208]

is important to note that this method is not specific for detecting those cells which do eventually undergo apoptosis and a more accurate means to detect actual cardiomyocyte apoptosis is by electron microscopy, but is rarely employed. A study of human diabetic myocardium has shown an 85-fold increase in cardiomyocyte apoptosis [159]. Such apoptotic myocyte loss may be one of the mechanisms contributing to the poor prognosis in diabetic patients after MI [161]. Several reports have shown that inhibition of myocardial cell death by antioxidants or inhibitors of apoptosis-specific signalling pathways, leads to significant prevention of diabetic cardiomyopathy [162]. Table 1 summarizes all of the structural changes which have been reported in diabetic cardiomyopathy.

### Myocardial microvessels

Although most structural studies of the myocardial vasculature have concentrated on coronary arteries and intramural arterioles, few studies have assessed myocardial capillary structure in either clinical or experimental diabetes. Some reports show significant ultrastructural changes in cardiomyocytes, in the absence of any vascular change [152,163], whereas others report vascular changes as the only specific myocardial alteration in the diabetic heart [157]. The aforementioned study by Thompson [153] was unable to detect any structural abnormalities in the myocardial capillaries during the first 6 weeks of diabetes. At 26 weeks, however, a 3-fold increase in thickening of the endothelial cytoplasm, with endothelial cell hypertrophy and cytoplasmic bridging led to complete obstruction of the lumen. The capillary basement membrane showed moderate, irregular thickening with occasional areas of duplication, with all changes distributed focally throughout the papillary muscle [153]. Thickening of the capillary basement membrane has been reported in mice [150], rats [164] and humans [157,165]. Thompson [166] further quantified the effect of diabetes on different components of the myocardial microvasculature in rats, by demonstrating a decrease

in mean capillary diameter by 11% after 12 weeks and 22% after 26 weeks of diabetes. Moreover, stereological analysis showed a significant progressive decrease in capillary density, which after 26 weeks of diabetes reached 77% of the value seen in non-diabetic age-matched control rats [166]. Ultrastructural signs of angiogenesis have also been reported and are characterized by alterations in endothelial and pericyte shape [164]. A study of human diabetic myocardium [157] found two characteristic abnormalities in myocardial capillaries: endothelial swelling and/or degeneration and thickening of the capillary basement membrane (Figure 2). This is the pathological hallmark of diabetic microangiopathy and occurred in an uneven, patchy, segmental manner [157]. Diabetic myocardial capillary microangiopathy has also been shown by others [167,168]. Table 2 summarizes the microvascular alterations seen in models of diabetic cardiomyopathy.

## TREATMENT

### Sulfonylureas

Of the relatively little published data on the use of sulfonylureas in diabetic HF, two studies have reported conflicting findings in terms of outcome [169,170]. A recent meta-analysis has indicated that these drugs are not associated with an increase in cardiovascular events [171,172]. Clearly, further studies are needed to determine the benefits and possible harm of these drugs in diabetic patients with HF.

### Metformin

Metformin was previously contraindicated in patients with HF in the U.S. due to the theoretical risk of lactic acidosis; its use is still strongly cautioned. Evidence to support the use of metformin in HF comes from three cohort studies in which metformin therapy was compared with other OHAs (oral hypoglycaemic agents)

**Table 2 Summary of the major microvascular changes in experimental and human diabetic cardiomyopathy**

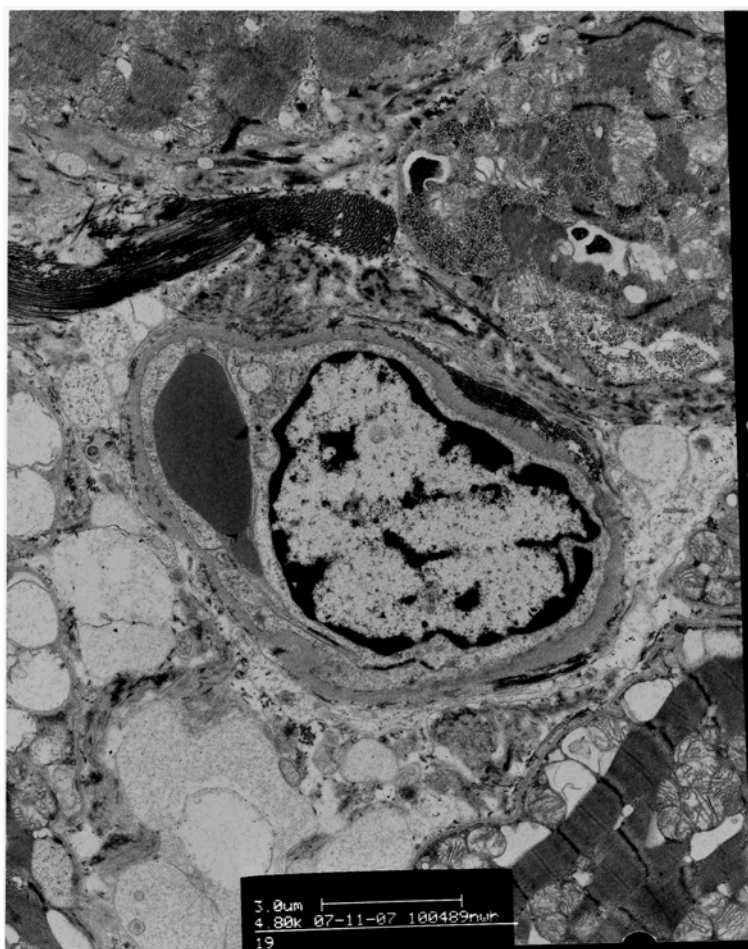
BB, BioBreeding; ZDF, Zucker diabetic fatty.

Diabetes type	Model	Microvascular changes
Type 1 diabetes	STZ rat	Decreased capillary diameter and density [166] Narrow capillary lumen with endothelial swelling [164] Basement membrane thickening [201]
	Alloxan rat	Decreased capillary lumen [153] Endothelial swelling [153] Basement membrane thickening [153]
	BB Wistar rat	No changes [152]
Type 2 diabetes	KK mice	No changes [163]
	ZDF	Perivascular fibrosis [202]
Type 1 and 2 diabetes	<i>ob/ob</i> Mice	Basement membrane thickening [150]
	Humans	Basement membrane thickening [157,165]

and insulin respectively [171]. Metformin treatment was associated with a reduction in all-cause mortality and all-cause hospital admissions. In addition to its modest insulin-sensitizing properties, metformin has been shown in an animal model to inhibit cardiac hypertrophy [173].

### TZDs

The TZDs [PPAR $\gamma$  (peroxisome-proliferator-activated receptor  $\gamma$ ) receptor agonists] are primarily insulin-sensitizing agents, but in addition to their antihyperglycaemic action, these drugs also exert beneficial effects on the myocardium, vascular endothelium, lipid profile and BP with additive anti-inflammatory and profibrinolytic effects [174]. They have been shown in both animal and human studies to improve myocardial metabolism and glucose handling [175]. In a 6 month double-blind, randomized, multicentre study comparing pioglitazone with glyburide in patients with Type 2 diabetes, systolic dysfunction and NYHA (New York Heart Association) functional class II/III HF, although



**Figure 2** Electronmicrograph of a myocardial capillary from a diabetic patient, demonstrating luminal occlusion with basement membrane thickening

pioglitazone was associated with a higher incidence of hospitalization for HF there was no increase in cardiovascular mortality or worsening cardiac function (by echocardiography) [176].

Thus the increased risk of HF is due to fluid retention via increased renal retention of sodium and subsequent plasma volume expansion and there is no evidence to suggest that TZDs may be harmful to the myocardium. In contrast, animal and experimental studies have demonstrated favourable effects on the myocardium such as reduced cardiac hypertrophy and improved systolic and diastolic function [177]. Results from clinical trials assessing TZD use and cardiac function however, is limited by both number and validity. Hence, the use of TZDs in HF is restricted to those in NYHA functional class I–II [178]. Two recent meta-analyses of seven and 19 randomized controlled trials of TZDs (pioglitazone or rosiglitazone), reported an increase in the risk of HF in diabetic and pre-diabetic subjects [RR of 1.72 and HR (hazard ratio) 1.41 respectively] [179,180], but there was no increase in the risk of cardiovascular mortality in these studies. Rosiglitazone has been associated with an increased risk of MI [177]. In the PROactive study, although pioglitazone was associated with a significant increase in serious HF (10.8% compared with 7.5% over 3 years,  $P < 0.0001$ ), there was no increase in mortality or cardiovascular events compared with placebo [181]. Thus the overall cardiovascular benefits derived from pioglitazone are thought to outweigh such risks [182]. In the RECORD (Rosaglitazone Evaluated for Cardiovascular Outcomes and Regulation of glycemia in Diabetes) study, rosiglitazone-treated patients had a slightly increased risk of incident HF (1.7 compared with 0.8%,  $P = 0.006$ ) after a mean treatment period of 3.8 years [183].

### Insulin

Insulin use in diabetic patients has also been associated with an increased risk of HF and death [171]. Cohort studies in diabetic populations with HF have yielded conflicting results. In the CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity) study, the risk of death was greater in diabetic patients treated with insulin compared with those not treated with insulin [184]. These findings contrast with those of UKPDS which did not report an increase in mortality with insulin use [171].

### GLP-1 (glucagon-like peptide-1) analogues

GLP-1 is an 'incretin' hormone which stimulates post-prandial insulin secretion and improves insulin sensitivity. GLP-1 analogues exert their cardiovascular benefit indirectly by inducing weight loss and improving glycaemic profile and endothelial function [185]. There is some promising data from a trial of GLP-1 analogues in patients following successful coronary intervention for acute MI whereby the treated group demonstrated a significantly

greater improvement in LVEF ( $29 \pm 2\%$  compared with  $39 \pm 2\%$ ,  $P < 0.01$ ) with concomitant improvements in global and regional wall motion [186]. The same group conducted a trial of GLP-1 analogues in patients with advanced HF and reported significant improvements in LVEF,  $\dot{V}O_{2\max}$  (maximal oxygen consumption), 6 min walk distance and standardized QOL (quality-of-life) score [187]. Clearly, long-term data are required for this class of drugs to determine their efficacy and safety in HF.

### DPP-4 (dipeptidyl peptidase-4) inhibitors

The DPP-4 inhibitors or gliptins are a group of drugs which increase incretin levels by inhibiting the enzyme DPP-4. Of note, DPP-4 is expressed on the endothelium of myocardial venules and paradoxically has been shown to be reduced in experimental diabetes [164]. These agents are relatively new and, as for the GLP-1 analogues, one may predict a long-term benefit on cardiovascular and all-cause mortality. Long-term morbidity and mortality trials are currently underway, but it will be several years before the data is reported.

### Metabolic modulators

These agents, previously used as anti-anginals, address the imbalance in myocyte energy efficiency by facilitating the switch from NEFA (non-esterified FA; 'free FA') to glucose metabolism. Trimetazidine, one such agent currently available in Europe, inhibits an enzyme involved in the  $\beta$ -oxidation of NEFAs and has been shown to improve LVEF, NYHA functional class and QOL in patients with HF [188]. Similarly, perhexiline has demonstrated substantial improvements in LVEF,  $\dot{V}O_{2\max}$  and QOL, but is associated with a risk of hepatotoxicity and peripheral neuropathy [189]. Ranolazine, is another drug with potential as a metabolic modulator [190]. It reduces intracellular  $\text{Na}^+$  and diastolic  $\text{Ca}^{2+}$  overload, thus improving diastolic function, but it has been associated with prolongation of the QT interval.

### The RAAS

The role of the RAAS in the pathogenesis of diabetic cardiomyopathy has been described above and forms the rationale for treatment with ACEIs and ARBs in diabetic cardiomyopathy. Both ACE inhibition and treatment with ARBs reduce total and cardiovascular mortality in diabetic patients [191,192]. In addition, there was a reduction in cardiovascular events in large studies such as RENAAL [45], HOPE (Heart Outcomes Prevention Evaluation) [193] and IDNT (Irbesartan Diabetic Nephropathy Trial) [194], the effect being more pronounced in diabetic compared with non-diabetic patients. Furthermore, in the CHARM study [184], candesartan was associated with a 40% reduction in the diagnosis of new onset diabetes and similarly, in the SOLVD study, treatment with enalapril reduced the

incidence of diabetes compared with placebo [23]. A meta-analysis of the major HF trials found an RR of 0.84 for death in diabetic patients treated with ACEIs [195]. The CHARM and Val-HeFT [196] studies also demonstrated mortality and morbidity benefits with ARBs which were similar in diabetic and non-diabetic patients.

### $\beta$ -Blockers

$\beta$ -Blockers are now well-established in the treatment of HF. Concerns in diabetic patients with regard to the increased risk of hypoglycaemia, dyslipidaemia and insulin resistance has meant that diabetic patients with HF are less likely to be discharged from hospital on a  $\beta$ -blocker than their non-diabetic counterparts [197]. However, the GEMINI (Clinical Utility of Amlodipine/Atorvastatin to Improve Concomitant Cardiovascular Risk Factors of Hypertension and Dyslipidemia) study compared the use of carvedilol with metoprolol in patients already receiving ACEIs or ARBs [198] and demonstrated that carvedilol improved insulin resistance and had no effect on HbA<sub>1c</sub>, whereas metoprolol worsened HbA<sub>1c</sub> and did not improve insulin resistance. Although to date there has not been a study of  $\beta$ -blockers in diabetic patients with HF, up to a quarter of patients in the major  $\beta$ -blocker trials in HF were diabetic [199,200]. Subgroup analysis of these trials demonstrated significant mortality and morbidity benefits in diabetic patients. In a meta-analysis of these trials, the overall RR of mortality in diabetic patients was 0.84 compared with 0.72 in non-diabetic patients.

### CONCLUSIONS

Considerable progress has been made to unravel the mechanistic basis for the increased frequency of HF in diabetic individuals. Functional consequences such as diastolic and then systolic dysfunction and failure correlate with glycaemic control. These functional alterations are preceded by a variety of structural, molecular and cellular changes, many of which are present in asymptomatic diabetic individuals and experimental models of diabetes. Moreover, alterations to myocardial and vascular integrity appear to initiate during a pre-diabetic stage. Although the aetiology of diabetic cardiomyopathy is complex and multifactorial, we have begun mapping a molecular, structural and functional basis for this condition and the debate regarding its existence as a distinct clinical entity is at an end. At present however, translational studies are lacking and it remains under-diagnosed and inadequately treated. Although hyperglycaemia appears to drive the pathogenesis of diabetic cardiomyopathy, intensive glycaemic control in diabetic patients has not translated into meaningful morbidity and mortality benefits, indeed recent results suggest the possibility of worsening outcomes. Novel diagnostic techniques to identify patients earlier, together with the development of more targeted therapeutic strategies ex-

ploiting recent experimental data, may lead to improved outcome in patients with diabetic cardiomyopathy.

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### REFERENCES

- Leyden, E. (1881) Asthma and diabetes mellitus. *Zeitschr. Klin. Med.* **3**, 358–364
- Mayer, J. (1888) Ueber den Zusammenhang des diabetes mellitus miterkrankungen des herzens. *Zeitschr. Klin. Med.* **14**, 212–239
- Rubler, S., Dlugash, J., Yuceoglu, Y. Z., Kumral, T., Branwood, A. W. and Grishman, A. (1972) New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am. J. Cardiol.* **30**, 595–602
- Hayat, S. A., Patel, B., Khattar, R. S. and Malik, R. A. (2004) Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. *Clin. Sci.* **107**, 539–557
- Thrainsdottir, I. S., Aspelund, T., Thorgeirsson, G., Gudnason, V., Hardarson, T., Malmberg, K., Sigurdsson, G. and Rydén, L. (2005) The association between glucose abnormalities and heart failure in the population-based Reykjavik study. *Diabetes Care* **28**, 612–616
- Bertoni, A. G., Hundley, W. G., Massing, M. W., Bonds, D. E., Burke, G. L. and Goff, Jr, D. C. (2004) Heart failure prevalence, incidence, and mortality in the elderly with diabetes. *Diabetes Care* **27**, 699–703
- Reis, S. E., Holubkov, R., Edmundowicz, D., McNamara, D. M., Zell, K. A., Detre, K. M. and Feldman, A. M. (1997) Treatment of patients admitted to the hospital with congestive heart failure: specialty-related disparities in practice patterns and outcomes. *J. Am. Coll. Cardiol.* **30**, 733–738
- Cohen-Solal, A., Beauvais, F. and Logeart, D. (2008) Heart failure and diabetes mellitus: epidemiology and management of an alarming association. *J. Card. Failure* **14**, 615–625
- Amato, L., Paolisso, G., Cacciatore, F., Ferrara, N., Ferrara, P., Canonic, S., Varricchio, M. and Rengo, F. (1997) Congestive heart failure predicts the development of non-insulin-dependent diabetes mellitus in the elderly. The Osservatorio Geriatrico Regione Campania Group. *Diabetes Metab.* **23**, 213–218
- Berry, C., Brett, M., Stevenson, K., McMurray, J. J. and Norrie, J. (2008) Nature and prognostic importance of abnormal glucose tolerance and diabetes in acute heart failure. *Heart* **94**, 296–304
- Mak, K. H., Moliterno, D. J., Granger, C. B., Miller, D. P., White, H. D., Wilcox, R. G., Califf, R. M. and Topol, E. J. (1997) Influence of diabetes mellitus on clinical outcome in the thrombolytic era of acute myocardial infarction. GUSTO-I Investigators. Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries. *J. Am. Coll. Cardiol.* **30**, 171–179
- Kannel, W. B., Hjortland, M. and Castelli, W. P. (1974) Role of diabetes in congestive heart failure: the Framingham study. *Am. J. Cardiol.* **34**, 29–34

- 13 Bertoni, A. G., Tsai, A., Kasper, E. K. and Brancati, F. L. (2003) Diabetes and idiopathic cardiomyopathy: a nationwide case-control study. *Diabetes Care* **26**, 2791–2795
- 14 Gottdiener, J. S., Arnold, A. M., Aurigemma, G. P., Polak, J. F., Tracy, R. P., Kitzman, D. W., Gardin, J. M., Rutledge, J. E. and Boineau, R. C. (2000) Predictors of congestive heart failure in the elderly: the Cardiovascular Health Study. *J. Am. Coll. Cardiol.* **35**, 1628–1637
- 15 Devereux, R. B., Roman, M. J., Paranicas, M., O'Grady, M. J., Lee, E. T., Welty, T. K., Fabsitz, R. R., Robbins, D., Rhoades, E. R. and Howard, B. V. (2000) Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation* **101**, 2271–2276
- 16 Bertoni, A. G., Goff, Jr, D. C., D'Agostino, Jr, R. B., Liu, K., Hundley, W. G., Lima, J. A., Polak, J. F., Saad, M. F., Szklo, M., Tracy, R. P. and Siscovick, D. S. (2006) Diabetic cardiomyopathy and subclinical cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* **29**, 588–594
- 17 Nichols, G. A., Hillier, T. A., Erbey, J. R. and Brown, J. B. (2001) Congestive heart failure in type 2 diabetes: prevalence, incidence, and risk factors. *Diabetes Care* **24**, 1614–1619
- 18 Aronow, W. S. and Ahn, C. (1999) Incidence of heart failure in 2737 older persons with and without diabetes mellitus. *Chest* **115**, 867–868
- 19 Stratton, I. M., Adler, A. I., Neil, H. A., Matthews, D. R., Manley, S. E., Cull, C. A., Hadden, D., Turner, R. C. and Holman, R. R. (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Br. Med. J.* **321**, 405–412
- 20 Iribarren, C., Karter, A. J., Go, A. S., Ferrara, A., Liu, J. Y., Sidney, S. and Selby, J. V. (2001) Glycemic control and heart failure among adult patients with diabetes. *Circulation* **103**, 2668–2673
- 21 Cheung, N., Wang, J. J., Rogers, S. L., Brancati, F., Klein, R., Sharrett, A. R. and Wong, T. Y. (2008) Diabetic retinopathy and risk of heart failure. *J. Am. Coll. Cardiol.* **51**, 1573–1578
- 22 Cheung, N., Bluemke, D. A., Klein, R., Sharrett, A. R., Islam, F. M., Cotch, M. F., Klein, B. E., Criqui, M. H. and Wong, T. Y. (2007) Retinal arteriolar narrowing and left ventricular remodeling: the multi-ethnic study of atherosclerosis. *J. Am. Coll. Cardiol.* **50**, 48–55
- 23 The SOLVD Investigators (1991) Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N. Engl. J. Med.* **325**, 293–302
- 24 Komajda, M., Wimart, M. C. and Thibout, E. (1994) The ATLAS study (Assessment of Treatment with Lisinopril and Survival); justification and objectives. *Arch. Mal. Coeur Vaiss* **87**, 45–50
- 25 Cohn, J. N., Archibald, D. G., Ziesche, S., Archibald, D. G., Ziesche, S., Francis, J. A., Harston, W. E., Tristani, F. E., Dunkman, W. B., Jacobs, W., Francis, G. S., Flohr, K. H. et al. (1986) Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. *N. Engl. J. Med.* **314**, 1547–1552
- 26 McKelvie, R. S., Yusuf, S., Pericak, D., Avezum, A., Burns, R. J., Probstfield, J., Tsuyuki, R. T., White, M., Rouleau, J., Latini, R. et al. (1999) Comparison of candesartan, enalapril, and their combination in congestive heart failure: randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study. The RESOLVD Pilot Study Investigators. *Circulation* **100**, 1056–1064
- 27 Ingelsson, E., Arnlov, J., Lind, L. and Sundstrom, J. (2006) Metabolic syndrome and risk for heart failure in middle-aged men. *Heart* **92**, 1409–1413
- 28 Ingelsson, E., Sundstrom, J., Arnlov, J., Zethelius, B. and Lind, L. (2005) Insulin resistance and risk of congestive heart failure. *JAMA, J. Am. Med. Assoc.* **294**, 334–341
- 29 Bahrami, H., Bluemke, D. A., Kronmal, R., Bertoni, A. G., Lloyd-Jones, D. M., Shahar, E., Szklo, M. and Lima, J. A. (2008) Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA (Multi-Ethnic Study of Atherosclerosis) study. *J. Am. Coll. Cardiol.* **51**, 1775–1783
- 30 Hassan, S. A., Deswal, A., Bozkurt, B., Aguilar, D., Mann, D. L. and Pritchett, A. M. (2008) The metabolic syndrome and mortality in an ethnically diverse heart failure population. *J. Card. Failure* **14**, 590–595
- 31 Andersen, N. H., Poulsen, S. H., Poulsen, P. L., Knudsen, S. T., Helleberg, K., Hansen, K. W., Dinesen, D. S., Eiskjaer, H., Flyvbjerg, A. and Mogensen, C. E. (2006) Effects of blood pressure lowering and metabolic control on systolic left ventricular function in Type II diabetes mellitus. *Clin. Sci.* **111**, 53–59
- 32 Fang, Z. Y., Schull-Meade, R., Downey, M., Prins, J. and Marwick, T. H. (2005) Determinants of subclinical diabetic heart disease. *Diabetologia* **48**, 394–402
- 33 Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R. and Neil, H. A. (2008) 10-Year follow-up of intensive glucose control in Type 2 diabetes. *N. Engl. J. Med.* **359**, 1577–1589
- 34 Patel, A., MacMahon, S., Chalmers, J., Neal, B., Billot, L., Woodward, M., Marre, M., Cooper, M., Glasziou, P., Grobbee, D. et al. (2008) Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med.* **358**, 2560–2572
- 35 Gerstein, H. C., Miller, M. E., Byington, R. P., Goff, Jr, D. C., Bigger, J. T., Buse, J. B., Cushman, W. C., Genuth, S., Ismail-Beigi, F., Grimm, Jr, R. H. et al. (2008) Effects of intensive glucose lowering in type 2 diabetes. *N. Engl. J. Med.* **358**, 2545–2559
- 36 Holman, R. R., Paul, S. K., Bethel, M. A., Neil, H. A. and Matthews, D. R. (2008) Long-term follow-up after tight control of blood pressure in Type 2 diabetes. *N. Engl. J. Med.* **359**, 1565–1576
- 37 Levy, D., Garrison, R. J., Savage, D. D., Kannel, W. B. and Castelli, W. P. (1990) Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N. Engl. J. Med.* **322**, 1561–1566
- 38 Yotsukura, M., Suzuki, J., Yamaguchi, T., Sasaki, K., Koide, Y., Mizuno, H., Yoshino, H. and Ishikawa, K. (1998) Prognosis following acute myocardial infarction in patients with ECG evidence of left ventricular hypertrophy prior to infarction. *J. Electrocardiol.* **31**, 91–99
- 39 Quinones, M. A., Greenberg, B. H., Kopelen, H. A., Koilpillai, C., Limacher, M. C., Shindler, D. M., Shelton, B. J. and Weiner, D. H. (2000) Echocardiographic predictors of clinical outcome in patients with left ventricular dysfunction enrolled in the SOLVD registry and trials: significance of left ventricular hypertrophy. *Studies of Left Ventricular Dysfunction. J. Am. Coll. Cardiol.* **35**, 1237–1244
- 40 Boner, G., Cooper, M. E., McCarroll, K., Brenner, B. M., de Zeeuw, D., Kowey, P. R., Shahinfar, S., Dickson, T., Crow, R. S. and Parving, H. H. (2005) Adverse effects of left ventricular hypertrophy in the reduction of endpoints in NIDDM with the angiotensin II antagonist losartan (RENAAL) study. *Diabetologia* **48**, 1980–1987
- 41 Lopez-Gomez, J. M., Verde, E. and Perez-Garcia, R. (1998) Blood pressure, left ventricular hypertrophy and long-term prognosis in hemodialysis patients. *Kidney Int. Suppl.* **68**, S92–S98
- 42 Eguchi, K., Ishikawa, J., Hoshida, S., Ishikawa, S., Pickering, T. G., Schwartz, J. E., Homma, S., Shimada, K. and Kario, K. (2007) Differential impact of left ventricular mass and relative wall thickness on cardiovascular prognosis in diabetic and nondiabetic hypertensive subjects. *Am. Heart J.* **154**, 79.e9–79.e15
- 43 de Simone, G., Gottdiener, J. S., Chinali, M. and Maurer, M. S. (2008) Left ventricular mass predicts heart failure not related to previous myocardial infarction: the Cardiovascular Health Study. *Eur. Heart J.* **29**, 741–747

- 44 Pierdomenico, S. D., Lapenna, D. and Cuccurullo, F. (2008) Regression of echocardiographic left ventricular hypertrophy after 2 years of therapy reduces cardiovascular risk in patients with essential hypertension. *Am. J. Hypertens.* **21**, 464–470
- 45 Brenner, B. M., Cooper, M. E., de Zeeuw, D., Keane, W. F., Mitch, W. E., Parving, H. H., Remuzzi, G., Snapinn, S. M., Zhang, Z. and Shahinfar, S. (2001) Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N. Engl. J. Med.* **345**, 861–869
- 46 Danielsen, R., Nordrehag, J. E., Lien, E. and Vik-Mo, H. (1987) Subclinical left ventricular abnormalities in young subjects with long-term type 1 diabetes mellitus detected by digitized M-mode echocardiography. *Am. J. Cardiol.* **60**, 143–146
- 47 Ozasa, N., Furukawa, Y., Morimoto, T., Tadamura, E., Kita, T. and Kimura, T. (2008) Relation among left ventricular mass, insulin resistance, and hemodynamic parameters in type 2 diabetes. *Hypertens. Res.* **31**, 425–432
- 48 Sundstrom, J., Arnlov, J., Stolare, K. and Lind, L. (2008) Blood pressure-independent relations of left ventricular geometry to the metabolic syndrome and insulin resistance: a population-based study. *Heart* **94**, 874–878
- 49 Zile, M. R., Baicu, C. F. and Gaasch, W. H. (2004) Diastolic heart failure: abnormalities in active relaxation and passive stiffness of the left ventricle. *N. Engl. J. Med.* **350**, 1953–1959
- 50 van Heerebeek, L., Hamdani, N., Handoko, M. L., Falcao-Pires, I., Musters, R. J., Kupreishvili, K., Ijsselmuiden, A. J., Schalkwijk, C. G., Bronzwaer, J. G., Diamant, M. et al. (2008) Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. *Circulation* **117**, 43–51
- 51 Karamitsos, T. D., Karvounis, H. I., Dalamanga, E. G., Papadopoulos, C. E., Didangelos, T. P., Karamitsos, D. T., Parharidis, G. E. and Louridas, G. E. (2007) Early diastolic impairment of diabetic heart: the significance of right ventricle. *Int. J. Cardiol.* **114**, 218–223
- 52 Zabalgoitia, M., Ismael, M. F., Anderson, L. and Maklady, F. A. (2001) Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. *Am. J. Cardiol.* **87**, 320–323
- 53 Boyer, J. K., Thanigaraj, S., Schechtman, K. B. and Perez, J. E. (2004) Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. *Am. J. Cardiol.* **93**, 870–875
- 54 Ha, J. W., Lee, H. C., Kang, E. S., Ahn, C. M., Kim, J. M., Ahn, J. A., Lee, S. W., Choi, E. Y., Rim, S. J., Oh, J. K. and Chung, N. (2007) Abnormal left ventricular longitudinal functional reserve in patients with diabetes mellitus: implication for detecting subclinical myocardial dysfunction using exercise tissue Doppler echocardiography. *Heart* **93**, 1571–1576
- 55 Svealy, B. G., Olofsson, E. L. and Andersson, B. (2008) Ventricular long-axis function is of major importance for long-term survival in patients with heart failure. *Heart* **94**, 284–289
- 56 Fang, Z. Y., Schull-Meade, R., Leano, R., Mottram, P. M., Prins, J. B. and Marwick, T. H. (2005) Screening for heart disease in diabetic subjects. *Am. Heart J.* **149**, 349–354
- 57 Fang, Z. Y., Yuda, S., Anderson, V., Short, L., Case, C. and Marwick, T. H. (2003) Echocardiographic detection of early diabetic myocardial disease. *J. Am. Coll. Cardiol.* **41**, 611–617
- 58 Yu, C. M., Lin, H., Yang, H., Kong, S. L., Zhang, Q. and Lee, S. W. (2002) Progression of systolic abnormalities in patients with 'isolated' diastolic heart failure and diastolic dysfunction. *Circulation* **105**, 1195–1201
- 59 Maciver, D. H. and Townsend, M. (2008) A novel mechanism of heart failure with normal ejection fraction. *Heart* **94**, 446–449
- 60 Aurigemma, G. P., Zile, M. R. and Gaasch, W. H. (2006) Contractile behavior of the left ventricle in diastolic heart failure: with emphasis on regional systolic function. *Circulation* **113**, 296–304
- 61 Fang, Z. Y., Leano, R. and Marwick, T. H. (2004) Relationship between longitudinal and radial contractility in subclinical diabetic heart disease. *Clin. Sci.* **106**, 53–60
- 62 Zornoff, L. A., Skali, H., Pfeffer, M. A., St John Sutton, M., Rouleau, J. L., Lamas, G. A., Plappert, T., Rouleau, J. R., Moyé, L. A., Lewis, S. J. et al. (2002) Right ventricular dysfunction and risk of heart failure and mortality after myocardial infarction. *J. Am. Coll. Cardiol.* **39**, 1450–1455
- 63 Zile, M. R., Gaasch, W. H., Carroll, J. D., Feldman, M. D., Aurigemma, G. P., Schaer, G. L., Ghali, J. K. and Liebson, P. R. (2001) Heart failure with a normal ejection fraction: is measurement of diastolic function necessary to make the diagnosis of diastolic heart failure? *Circulation* **104**, 779–782
- 64 Galderisi, M. (2006) Diastolic dysfunction and diabetic cardiomyopathy: evaluation by Doppler echocardiography. *J. Am. Coll. Cardiol.* **48**, 1548–1551
- 65 Edvardsen, T., Gerber, B. L., Garot, J., Bluemke, D. A., Lima, J. A. and Smiseth, O. A. (2002) Quantitative assessment of intrinsic regional myocardial deformation by Doppler strain rate echocardiography in humans: validation against three-dimensional tagged magnetic resonance imaging. *Circulation* **106**, 50–56
- 66 Gotte, M. J., van Rossum, A. C., Twisk, J. W. R., Kuijjer, J. P. A., Marcus, J. T. and Visser, C. A. (2001) Quantification of regional contractile function after infarction: strain analysis superior to wall thickening analysis in discriminating infarct from remote myocardium. *J. Am. Coll. Cardiol.* **37**, 808–817
- 67 Chuang, M. L., Beaudin, R. A., Riley, M. F., Mooney, M. G., Mannin, W. J., Douglas, P. S. and Hibberd, M. G. (2000) Three-dimensional echocardiographic measurement of left ventricular mass: comparison with magnetic resonance imaging and two-dimensional echocardiographic determinations in man. *Int. J. Cardiac Imaging* **16**, 347–357
- 68 Maisel, A. S., McCord, J., Nowak, R. M., Hollander, J. E., Wu, A. H., Duc, P., Omland, T., Storrow, A. B., Krishnaswamy, P., Abraham, W. T. et al. (2003) Bedside B-type natriuretic peptide in the emergency diagnosis of heart failure with reduced or preserved ejection fraction. Results from the Breathing Not Properly Multinational Study. *J. Am. Coll. Cardiol.* **41**, 2010–2017
- 69 Shen, X. and Bornfeldt, K. E. (2007) Mouse models for studies of cardiovascular complications of type 1 diabetes. *Ann. N. Y. Acad. Sci.* **1103**, 202–217
- 70 Retnakaran, R. and Zinman, B. (2008) Type 1 diabetes, hyperglycaemia, and the heart. *Lancet* **371**, 1790–1799
- 71 Wold, L. E., Ceylan-Isik, A. F., Fang, C. X., Yang, X., Li, S. Y., Sreejayan, N., Privratsky, J. R. and Ren, J. (2006) Metallothionein alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of Ca<sup>2+</sup> cycling proteins, NADPH oxidase, poly(ADP-ribose) cyclase and myosin heavy chain isoforms. *Free Radical Biol. Med.* **40**, 1419–1429
- 72 Liang, Q., Carlson, E. C., Donthi, R. V., Kralik, P. M., Shen, X. and Epstein, P. N. (2002) Overexpression of metallothionein reduces diabetic cardiomyopathy. *Diabetes* **51**, 174–181
- 73 Cai, L., Wang, Y., Zhou, G., Chen, T., Song, Y., Li, X. and Kang, Y. J. (2006) Attenuation by metallothionein of early cardiac cell death via suppression of mitochondrial oxidative stress results in a prevention of diabetic cardiomyopathy. *J. Am. Coll. Cardiol.* **48**, 1688–1697
- 74 Ye, G., Metreveli, N. S., Donthi, R. V., Xia, S., Xu, M., Carlson, E. C. and Epstein, P. N. (2004) Catalase protects cardiomyocyte function in models of type 1 and type 2 diabetes. *Diabetes* **53**, 1336–1343
- 75 Seddon, M., Looi, Y. H. and Shah, A. M. (2007) Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. *Heart* **93**, 903–907

- 76 Gao, W. D., Liu, Y. and Marban, E. (1996) Selective effects of oxygen free radicals on excitation-contraction coupling in ventricular muscle. Implications for the mechanism of stunned myocardium. *Circulation* **94**, 2597–2604
- 77 Li, J.-M., Gall, N. P., Grieve, D. J., Chen, M. and Shah, A. M. (2002) Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* **40**, 477–484
- 78 Wold, L. E., Ceylan-Isik, A. F., Fang, C. X., Yang, X., Li, S. Y., Sreejayan, N., Privratsky, J. R. and Ren, J. (2006) Metallothionein alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of  $\text{Ca}^{2+}$  cycling proteins, NADPH oxidase, poly(ADP-ribose) polymerase and myosin heavy chain isozyme. *Free Radical Biol. Med.* **40**, 1419–1429
- 79 Ritchie, R. H., Quinn, J. M., Cao, A. H., Drummond, G. R., Kaye, D. M., Favalaro, J. M., Proietto, J. and Delbridge, L. M. (2007) The antioxidant tempol inhibits cardiac hypertrophy in the insulin-resistant GLUT4-deficient mouse *in vivo*. *J. Mol. Cell. Cardiol.* **42**, 1119–1128
- 80 Tesfamariam, B., Brown, M. L. and Cohen, R. A. (1991) Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J. Clin. Invest.* **87**, 1643–1648
- 81 Duncan, E. R., Walker, S. J., Ezzat, V. A., Wheatcroft, S. B., Li, J. M., Shah, A. M. and Kearney, M. T. (2007) Accelerated endothelial dysfunction in mild prediabetic insulin resistance: the early role of reactive oxygen species. *Am. J. Physiol. Endocrinol. Metab.* **293**, E1311–E1319
- 82 Hornig, B., Arakawa, N., Kohler, C. and Drexler, H. (1998) Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* **97**, 363–368
- 83 MacCarthy, P. A., Grieve, D. J., Li, J. M., Dunster, C., Kelly, F. J. and Shah, A. M. (2001) Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy: role of reactive oxygen species and NADPH oxidase. *Circulation* **104**, 2967–2974
- 84 Yusuf, S., Dagenais, G., Pogue, J., Bosch, J. and Sleight, P. (2000) Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N. Engl. J. Med.* **342**, 154–160
- 85 Du, X., Matsumura, T., Edelstein, D., Rossetti, L., Zsengeller, Z., Szabó, C. and Brownlee, M. (2003) Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J. Clin. Invest.* **112**, 1049–1057
- 86 Reference deleted
- 87 Szabo, C. (2002) PARP as a drug target for the therapy of diabetic cardiovascular dysfunction. *Drug News Perspect.* **15**, 197–205
- 88 Minchenko, A. G., Stevens, M. J., White, L., Abatan, O. I., Komjáti, K., Pacher, P., Szabó, C. and Obrosova, I. G. (2003) Diabetes-induced overexpression of endothelin-1 and endothelin receptors in the rat renal cortex is mediated via poly(ADP-ribose) polymerase activation. *FASEB J.* **17**, 1514–1516
- 89 Chiu, J., Farhangkhoe, H., Xu, B. Y., Chen, S., George, B. and Chakrabarti, S. (2008) PARP mediates structural alterations in diabetic cardiomyopathy. *J. Mol. Cell. Cardiol.* **45**, 385–393
- 90 Yang, Z., Zingarelli, B. and Szabo, C. (2000) Effect of genetic disruption of poly (ADP-ribose) synthetase on delayed production of inflammatory mediators and delayed necrosis during myocardial ischemia-reperfusion injury. *Shock* **13**, 60–66
- 91 Soriano, F. G., Pacher, P., Mabley, J., Liaudet, L. and Szabo, C. (2001) Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. *Circ. Res.* **89**, 684–691
- 92 Bowling, N., Walsh, R. A., Song, G., Estridge, T., Sandusky, G. E., Fouts, R. L., Mintze, K., Pickard, T., Roden, R., Bristow, M. R. et al. (1999) Increased protein kinase C activity and expression of  $\text{Ca}^{2+}$ -sensitive isoforms in the failing human heart. *Circulation* **99**, 384–391
- 93 Way, K. J., Isshiki, K., Suzuma, K., Yokota, T., Zvagelsky, D., Schoen, F. J., Sandusky, G. E., Pechous, P. A., Vlahos, C. J., Wakasaki, H. and King, G. L. (2002) Expression of connective tissue growth factor is increased in injured myocardium associated with protein kinase C  $\beta 2$  activation and diabetes. *Diabetes* **51**, 2709–2718
- 94 Koya, D. and King, G. L. (1998) Protein kinase C activation and the development of diabetic complications. *Diabetes* **47**, 859–866
- 95 Braz, J. C., Gregory, K., Pathak, A., Zhao, W., Sahin, B., Klevitsky, R., Kimball, T. F., Lorenz, J. N., Nairn, A. C., Liggett, S. B. et al. (2004) PKC $\alpha$  regulates cardiac contractility and propensity toward heart failure. *Nat. Med.* **10**, 248–254
- 96 Wakasaki, H., Koya, D., Schoen, F. J., Jirousek, M. R., Ways, D. K., Hoit, B. D., Walsh, R. A. and King, G. L. (1997) Targeted overexpression of protein kinase C  $\beta 2$  isoform in myocardium causes cardiomyopathy. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9320–9325
- 97 Topham, M. K. and Prescott, S. M. (2002) Diacylglycerol kinases: regulation and signaling roles. *Thrombosis Haemostasis* **88**, 912–918
- 98 Harada, M., Takeishi, Y., Arimoto, T., Niizeki, T., Kitahara, T., Goto, K., Walsh, R. A. and Kubota, I. (2007) Diacylglycerol kinase  $\zeta$  attenuates pressure overload-induced cardiac hypertrophy. *Circ. J.* **71**, 276–282
- 99 Bilim, O., Takeishi, Y., Kitahara, T., Arimoto, T., Niizeki, T., Sasaki, T., Goto, K. and Kubota, I. (2008) Diacylglycerol kinase  $\zeta$  inhibits myocardial atrophy and restores cardiac dysfunction in streptozotocin-induced diabetes mellitus. *Cardiovasc. Diabetol.* **7**, 2
- 100 Xia, Z., Kuo, K.-H., Nagareddy, P. R., Wang, F., Guo, Z., Guo, T., Jiang, J. and McNeill, J. H. (2007) *N*-acetylcysteine attenuates PKC $\beta 2$  overexpression and myocardial hypertrophy in streptozotocin-induced diabetic rats. *Cardiovasc. Res.* **73**, 770–782
- 101 Hambleton, M., Hahn, H., Pleger, S. T., Kuhn, M. C., Klevitsky, R., Carr, A. N., Kimball, T. F., Hewett, T. E., Dorn, 2nd, G. W., Koch, W. J. and Molkenin, J. D. (2006) Pharmacological- and gene therapy-based inhibition of protein kinase C $\alpha/\beta$  enhances cardiac contractility and attenuates heart failure. *Circulation* **114**, 574–582
- 102 Arikawa, E., Ma, R. C. W., Isshiki, K., Luptak, I., He, Z., Yasuda, Y., Maeno, Y., Patti, M. E., Weir, G. C., Harris, R. A. et al. (2007) Effects of insulin replacements, inhibitors of angiotensin, and PKC $\beta$ 's actions to normalize cardiac gene expression and fuel metabolism in diabetic rats. *Diabetes* **56**, 1410–1420
- 103 Montagnani, M. (2008) Diabetic cardiomyopathy: how much does it depend on AGE? *Br. J. Pharmacol.* **154**, 725–726
- 104 Aragno, M., Mastrocola, R., Medana, C., Catalano, M. G., Vercellinato, I., Danni, O. and Boccuzzi, G. (2006) Oxidative stress-dependent impairment of cardiac-specific transcription factors in experimental diabetes. *Endocrinology* **147**, 5967–5974
- 105 Carley, A. N. and Severson, D. L. (2008) What are the biochemical mechanisms responsible for enhanced fatty acid utilization by perfused hearts from type 2 diabetic *db/db* mice? *Cardiovasc. Drugs Ther.* **22**, 83–89
- 106 Bers, D. M. (2002) Cardiac excitation-contraction coupling. *Nature* **415**, 198–205
- 107 Trost, S. U., Belke, D. D., Bluhm, W. F., Meyer, M., Swanson, E. and Dillmann, W. H. (2002) Overexpression of the sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase improves myocardial contractility in diabetic cardiomyopathy. *Diabetes* **51**, 1166–1171



- 108 Hattori, Y., Matsuda, N., Kimura, J., Ishitani, T., Tamada, A., Gando, S., Kemmotsu, O. and Kanno, M. (2000) Diminished function and expression of the cardiac  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger in diabetic rats: implication in  $\text{Ca}^{2+}$  overload. *J. Physiol.* **527**, 85–94
- 109 Pereira, L., Matthes, J., Schuster, I., Valdivia, H. H., Herzig, S., Richard, S. and Gómez, A. M. (2006) Mechanisms of  $[\text{Ca}^{2+}]_i$  transient decrease in cardiomyopathy of *db/db* type 2 diabetic mice. *Diabetes* **55**, 608–615
- 110 Golfman, L., Dixon, I. M., Takeda, N., Lukas, A., Dakshinamurti, K. and Dhalla, N. S. (1998) Cardiac sarcolemmal  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange and  $\text{Na}^+$ - $\text{K}^+$  ATPase activities and gene expression in alloxan-induced diabetes in rats. *Mol. Cell. Biochem.* **188**, 91–101
- 111 Vetter, R., Rehfeld, U., Reissfelder, C., Weiss, W., Wagner, K. D., Günther, J., Hammes, A., Tschöpe, C., Dillmann, W. and Paul, M. (2002) Transgenic overexpression of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  ATPase improves reticular  $\text{Ca}^{2+}$  handling in normal and diabetic rat hearts. *FASEB J.* **16**, 1657–1659
- 112 Yaras, N., Bilginoglu, A., Vassort, G. and Turan, B. (2007) Restoration of diabetes-induced abnormal local  $\text{Ca}^{2+}$  release in cardiomyocytes by angiotensin II receptor blockade. *Am. J. Physiol. Heart Circ. Physiol.* **292**, H912–H920
- 113 Shishebor, M. H., Hoogwerf, B. J., Schoenhagen, P., Marso, S. P., Sun, J. P., Li, J., Klein, A. L., Thomas, J. D. and Garcia, M. J. (2003) Relation of hemoglobin A1c to left ventricular relaxation in patients with type 1 diabetes mellitus and without overt heart disease. *Am. J. Cardiol.* **91**, 1514–1517
- 114 Jweied, E. E., McKinney, R. D., Walker, L. A., Brodsky, I., Geha, A. S., Massad, M. G., Buttrick, P. M. and de Tombe, P. P. (2005) Depressed cardiac myofilament function in human diabetes mellitus. *Am. J. Physiol. Heart Circ. Physiol.* **289**, H2478–H2483
- 115 Qin, D., Huang, B., Deng, L., El-Adawi, H., Ganguly, K., Sowers, J. R. and El-Sherif, N. (2001) Downregulation of  $\text{K}^+$  channel genes expression in type I diabetic cardiomyopathy. *Biochem. Biophys. Res. Commun.* **283**, 549–553
- 116 Dostal, D. E., Rothblum, K. N., Chernin, M. I., Cooper, G. R. and Baker, K. M. (1992) Intracardiac detection of angiotensinogen and renin: a localized renin-angiotensin system in neonatal rat heart. *Am. J. Physiol.* **263**, C838–C850
- 117 Privratsky, J. R., Wold, L. E., Sowers, J. R., Quinn, M. T. and Ren, J. (2003) AT1 blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: role of the AT1 receptor and NADPH oxidase. *Hypertension* **42**, 206–212
- 118 Connelly, K. A., Kelly, D. J., Zhang, Y., Prior, D. L., Martin, J., Cox, A. J., Thai, K., Feneley, M. P., Tsoporis, J., White, K. E. et al. (2007) Functional, structural and molecular aspects of diastolic heart failure in the diabetic (mRen-2)27 rat. *Cardiovasc. Res.* **76**, 280–291
- 119 Raimondi, L., De Paoli, P., Mannucci, E., Lonardo, G., Sartiani, L., Banchelli, G., Pirisino, R., Mugelli, A. and Cerbai, E. (2004) Restoration of cardiomyocyte functional properties by angiotensin II receptor blockade in diabetic rats. *Diabetes* **53**, 1927–1933
- 120 Fiordaliso, F., Cuccovillo, I., Bianchi, R., Bai, A., Doni, M., Salio, M., De Angelis, N., Ghezzi, P., Latini, R. and Masson, S. (2006) Cardiovascular oxidative stress is reduced by an ACE inhibitor in a rat model of streptozotocin-induced diabetes. *Life Sci.* **79**, 121–129
- 121 Liu, X., Suzuki, H., Sethi, R., Tappia, P. S., Takeda, N. and Dhalla, N. S. (2006) Blockade of the renin-angiotensin system attenuates sarcolemma and sarcoplasmic reticulum remodeling in chronic diabetes. *Ann. N. Y. Acad. Sci.* **1084**, 141–154
- 122 Heart Outcomes Prevention Evaluation Study Investigators (2000) Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* **355**, 253–259
- 123 Zannad, F., Alla, F., Dousset, B., Perez, A. and Pitt, B. (2000) Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). *Circulation* **102**, 2700–2706
- 124 McEwan, P. E., Gray, G. A., Sherry, L., Webb, D. J. and Kenyon, C. J. (1998) Differential effects of angiotensin II on cardiac cell proliferation and intramyocardial perivascular fibrosis *in vivo*. *Circulation* **98**, 2765–2773
- 125 Neumann, S., Huse, K., Semrau, R., Diegeler, A., Gebhardt, R., Bunatian, G. H. and Scholz, G. H. (2002) Aldosterone and D-glucose stimulate the proliferation of human cardiac myofibroblasts *in vitro*. *Hypertension* **39**, 756–760
- 126 Liu, Y., Cox, S. R., Morita, T. and Kourembanas, S. (1995) Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. *Circ. Res.* **77**, 638–643
- 127 Richard, D. E., Berra, E. and Pouyssegur, J. (2000) Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1 $\alpha$  in vascular smooth muscle cells. *J. Biol. Chem.* **275**, 26765–26771
- 128 Abaci, A., Oguzhan, A., Kahraman, S., Eryol, N. K., Unal, S., Arinc, H. and Ergin, A. (1999) Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation* **99**, 2239–2242
- 129 Shinohara, K., Shinohara, T., Mochizuki, N., Mochizuki, Y., Sawa, H., Kohya, T., Fujita, M., Fujioka, Y., Kitabatake, A. and Nagashima, K. (1996) Expression of vascular endothelial growth factor in human myocardial infarction. *Heart Vessels* **11**, 113–122
- 130 Chou, E., Suzuma, I., Way, K. J., Opland, D., Clermont, A. C., Naruse, K., Suzuma, K., Bowling, N. L., Vlahos, C. J., Aiello, L. P. and King, G. L. (2002) Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. *Circulation* **105**, 373–379
- 131 Emoto, M., Anno, T., Sato, Y., Tanabe, K., Okuya, S., Tanizawa, Y., Matsutani, A. and Oka, Y. (2001) Troglitazone treatment increases plasma vascular endothelial growth factor in diabetic patients and its mRNA in 3T3-L1 adipocytes. *Diabetes* **50**, 1166–1170
- 132 Ebrahimian, T. G., Heymes, C., You, D., Blanc-Brude, O., Mees, B., Waackel, L., Duriez, M., Vilar, J., Brandes, R. P., Levy, B. I. et al. (2006) NADPH oxidase-derived overproduction of reactive oxygen species impairs postischemic neovascularization in mice with type 1 diabetes. *Am. J. Pathol.* **169**, 719–728
- 133 Jesmin, S., Zaedi, S., Shimojo, N., Iemitsu, M., Masuzawa, K., Yamaguchi, N., Mowa, C. N., Maeda, S., Hattori, Y. and Miyauchi, T. (2007) Endothelin antagonism normalizes VEGF signaling and cardiac function in STZ-induced diabetic rat hearts. *Am. J. Physiol. Endocrinol. Metab.* **292**, E1030–E1040
- 134 Tschöpe, C., Schultheiss, H. P., Walther, T., Tschöpe, C., Schultheiss, H.-P. and Walther, T. (2002) Multiple interactions between the renin-angiotensin and the kallikrein-kinin systems: role of ACE inhibition and AT1 receptor blockade. *J. Cardiovasc. Pharmacol.* **39**, 478–487
- 135 Tschöpe, C. and Westermann, D. (2008) Development of diabetic cardiomyopathy and the kallikrein-kinin system—new insights from B1 and B2 receptor signaling. *Biol. Chem.* **389**, 707–711
- 136 Spillmann, F., Van Linthout, S., Schultheiss, H. P. and Tschöpe, C. (2006) Cardioprotective mechanisms of the kallikrein-kinin system in diabetic cardiomyopathy. *Curr. Opin. Nephrol. Hypertension* **15**, 22–29

- 137 Cloutier, F. and Couture, R. (2000) Pharmacological characterization of the cardiovascular responses elicited by kinin B(1) and B(2) receptor agonists in the spinal cord of streptozotocin-diabetic rats. *Br. J. Pharmacol.* **130**, 375–385
- 138 Tschöpe, C., Walther, T., Yu, M., Reinecke, A., Koch, M., Seligmann, C., Heringer, S. B., Pesquero, J. B., Bader, M., Schultheiss, H. and Unger, T. (1999) Myocardial expression of rat bradykinin receptors and two tissue kallikrein genes in experimental diabetes. *Immunopharmacology* **44**, 35–42
- 139 Pelikanova, T., Pinsker, P., Smrckova, I., Stribrna, L. and Dryakova, M. (1998) Decreased urinary kallikrein with hyperglycemia in patients with short-term insulin-dependent diabetes mellitus. *J. Diabetes Compl.* **12**, 264–272
- 140 Rett, K., Wicklmayr, M., Dietze, G. J. and Haring, H. U. (1996) Insulin-induced glucose transporter (GLUT1 and GLUT4) translocation in cardiac muscle tissue is mimicked by bradykinin. *Diabetes* **45** (Suppl. 1), S66–S69
- 141 Shiuchi, T., Cui, T. X., Wu, L., Nakagami, H., Takeda-Matsubara, Y., Iwai, M. and Horiuchi, M. (2002) ACE inhibitor improves insulin resistance in diabetic mouse via bradykinin and NO. *Hypertension* **40**, 329–334
- 142 Montanari, D., Yin, H., Dobrzynski, E., Agata, J., Yoshida, H., Chao, J. and Chao, L. (2005) Kallikrein gene delivery improves serum glucose and lipid profiles and cardiac function in streptozotocin-induced diabetic rats. *Diabetes* **54**, 1573–1580
- 143 Tschöpe, C., Walther, T., Escher, F., Spillmann, F., Du, J., Altmann, C., Schimke, I., Bader, M., Sanchez-Ferrer, C. F., Schultheiss, H. P. and Noutsias, M. (2005) Transgenic activation of the kallikrein-kinin system inhibits intramyocardial inflammation, endothelial dysfunction and oxidative stress in experimental diabetic cardiomyopathy. *FASEB J.* **19**, 2057–2059
- 144 Mikrut, K., Paluszak, J., Kozlik, J., Sosnowski, P., Krauss, H. and Grzeskowiak, E. (2001) The effect of bradykinin on the oxidative state of rats with acute hyperglycaemia. *Diabetes Res. Clin. Prac.* **51**, 79–85
- 145 Tschöpe, C., Spillmann, F., Rehfeld, U., Koch, M., Westermann, D., Altmann, C., Dendorfer, A., Walther, T., Bader, M., Paul, M. et al. (2004) Improvement of defective sarcoplasmic reticulum Ca<sup>2+</sup> transport in diabetic heart of transgenic rats expressing the human kallikrein-1 gene. *FASEB J.* **18**, 1967–1969
- 146 Tschöpe, C., Walther, T., Koniger, J., Spillmann, F., Westermann, D., Escher, F., Pauschinger, M., Pesquero, J. B., Bader, M., Schultheiss, H. P. and Noutsias, M. (2004) Prevention of cardiac fibrosis and left ventricular dysfunction in diabetic cardiomyopathy in rats by transgenic expression of the human tissue kallikrein gene. *FASEB J.* **18**, 828–835
- 147 Regan, T. J., Wu, C. F., Yeh, C. K., Oldewurtel, H. A. and Haider, B. (1981) Myocardial composition and function in diabetes. The effects of chronic insulin use. *Circ. Res.* **49**, 1268–1277
- 148 Haider, B., Yeh, C. K., Thomas, G., Oldewurtel, H. A., Lyons, M. M. and Regan, T. J. (1981) Influence of diabetes on the myocardium and coronary arteries of rhesus monkey fed an atherogenic diet. *Circ. Res.* **49**, 1278–1288
- 149 Bhimji, S., Godin, D. V. and McNeill, J. H. (1986) Myocardial ultrastructural changes in alloxan-induced diabetes in rabbits. *Acta Anat. (Basel)* **125**, 195–200
- 150 Giacomelli, F. and Wiener, J. (1979) Primary myocardial disease in the diabetic mouse. An ultrastructural study. *Lab. Invest.* **40**, 460–473
- 151 Jackson, C. V., McGrath, G. M., Tahiliani, A. G., Vadlamudi, R. V. and McNeill, J. H. (1985) A functional and ultrastructural analysis of experimental diabetic rat myocardium. Manifestation of a cardiomyopathy. *Diabetes* **34**, 876–883
- 152 Hsiao, Y. C., Suzuki, K., Abe, H. and Toyota, T. (1987) Ultrastructural alterations in cardiac muscle of diabetic BB Wistar rats. *Virchows Arch. A Pathol. Anat. Histopathol.* **411**, 45–52
- 153 Thompson, E. W. (1988) Structural manifestations of diabetic cardiomyopathy in the rat and its reversal by insulin treatment. *Am. J. Anat.* **182**, 270–282
- 154 Tarach, J. S. (1976) Histomorphological and ultrastructural studies of a rat myocardium in experimental conditions. *Z. Mikrosk. Anat. Forsch.* **90**, 1145–1157
- 155 Reinila, A. and Akerblom, H. K. (1984) Ultrastructure of heart muscle in short-term diabetic rats: influence of insulin treatment. *Diabetologia* **27**, 397–402
- 156 Koltai, M. Z., Balogh, I., Wagner, M. and Pogatsa, G. (1984) Diabetic myocardial alterations in ultrastructure and function. *Exp. Pathol.* **25**, 215–221
- 157 Fischer, V. W., Barner, H. B. and Larose, L. S. (1984) Pathomorphologic aspects of muscular tissue in diabetes mellitus. *Hum. Pathol.* **15**, 1127–1136
- 158 Dyntar, D., Eppenberger-Eberhardt, M., Maedler, K., Pruschy, M., Eppenberger, H. M., Spinass, G. A. and Donath, M. Y. (2001) Glucose and palmitic acid induce degeneration of myofibrils and modulate apoptosis in rat adult cardiomyocytes. *Diabetes* **50**, 2105–2113
- 159 Frustaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., Nadal-Ginard, B. and Anversa, P. (2000) Myocardial cell death in human diabetes. *Circ. Res.* **87**, 1123–1132
- 160 Dyntar, D., Sergeev, P., Klisic, E., Ambuhl, P., Schaub, M. C. and Donath, M. Y. (2006) High glucose alters cardiomyocyte contacts and inhibits myofibrillar formation. *J. Clin. Endocrinol. Metab.* **91**, 1961–1967
- 161 Backlund, T., Palojoki, E., Saraste, A., Eriksson, A., Finckenberg, P., Kytö, V., Lakkisto, P., Mervaala, E., Voipio-Pulkki, L. M., Laine, M. and Tikkanen, I. (2004) Sustained cardiomyocyte apoptosis and left ventricular remodelling after myocardial infarction in experimental diabetes. *Diabetologia* **47**, 325–330
- 162 Cai, L. and Kang, Y. J. (2003) Cell death and diabetic cardiomyopathy. *Cardiovasc. Toxicol.* **3**, 219–228
- 163 Saito, K., Nishi, S., Kashima, T. and Tanaka, H. (1984) Histologic and ultrastructural studies on the myocardium in spontaneously diabetic KK mice: a new animal model of cardiomyopathy. *Am. J. Cardiol.* **53**, 320–323
- 164 Okruhlicova, L., Tribulova, N., Weismann, P. and Sotnikova, R. (2005) Ultrastructure and histochemistry of rat myocardial capillary endothelial cells in response to diabetes and hypertension. *Cell Res.* **15**, 532–538
- 165 Silver, M. D., Huckell, V. F. and Lorber, M. (1977) Basement membranes of small cardiac vessels in patients with diabetes and myxoedema: preliminary observations. *Pathology* **9**, 213–220
- 166 Thompson, E. W. (1994) Quantitative analysis of myocardial structure in insulin-dependent diabetes mellitus: effects of immediate and delayed insulin replacement. *Proc. Soc. Exp. Biol. Med.* **205**, 294–305
- 167 Cameron, D. P., Amherdt, M., Leuenberger, P., Orci, L. and Stauffacher, W. (1973) Microvascular alterations in chronically streptozotocin-diabetic rats. *Adv. Metab. Disord.* **2** (Suppl. 2), 257–269
- 168 Kilo, C., Vogler, N. and Williamson, J. R. (1972) Muscle capillary basement membrane changes related to aging and to diabetes mellitus. *Diabetes* **21**, 881–905
- 169 Eurich, D. T., Majumdar, S. R., McAlister, F. A., Tsuyuki, R. T. and Johnson, J. A. (2005) Improved clinical outcomes associated with metformin in patients with diabetes and heart failure. *Diabetes Care* **28**, 2345–2351
- 170 Masoudi, F. A., Inzucchi, S. E., Wang, Y., Havranek, E. P., Foody, J. M. and Krumholz, H. M. (2005) Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. *Circulation* **111**, 583–590
- 171 Eurich, D. T., McAlister, F. A., Blackburn, D. F., Majumdar, S. R., Tsuyuki, R. T., Varney, J. and Johnson, J. A. (2007) Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review. *Br. Med. J.* **335**, 497

- 172 Gangji, A. S., Cukierman, T., Gerstein, H. C., Goldsmith, C. H. and Clase, C. M. (2007) A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care* **30**, 389–394
- 173 Chan, A. Y., Soltys, C. L., Young, M. E., Proud, C. G. and Dyck, J. R. (2004) Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. *J. Biol. Chem.* **279**, 32771–32779
- 174 McGuire, D. K. and Inzucchi, S. E. (2008) New drugs for the treatment of diabetes mellitus: part I: thiazolidinediones and their evolving cardiovascular implications. *Circulation* **117**, 440–449
- 175 Hallsten, K., Virtanen, K. A., Lonqvist, F., Janatuinen, T., Turiceanu, M., Rönnemaa, T., Viikari, J., Lehtimäki, T., Knuuti, J. and Nuutila, P. (2004) Enhancement of insulin-stimulated myocardial glucose uptake in patients with Type 2 diabetes treated with rosiglitazone. *Diabetes Med.* **21**, 1280–1287
- 176 Giles, T. D., Miller, A. B., Elkayam, U., Bhattacharya, M. and Perez, A. (2008) Pioglitazone and heart failure: results from a controlled study in patients with type 2 diabetes mellitus and systolic dysfunction. *J. Card. Failure* **14**, 445–452
- 177 Nissen, S. E. and Wolski, K. (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N. Engl. J. Med.* **356**, 2457–2471
- 178 St John Sutton, M., Rendell, M., Dandona, P., Dole, J. F., Murphy, K., Patwardhan, R., Patel, J. and Freed, M. (2002) A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycemic control in patients with type 2 diabetes. *Diabetes Care* **25**, 2058–2064
- 179 Lago, R. M., Singh, P. P. and Nesto, R. W. (2007) Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. *Lancet* **370**, 1129–1136
- 180 Lincoff, A. M., Wolski, K., Nicholls, S. J. and Nissen, S. E. (2007) Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. *JAMA, J. Am. Med. Assoc.* **298**, 1180–1188
- 181 Dormandy, J. A., Charbonnel, B., Eckland, D. J., Erdmann, E., Massi-Benedetti, M., Moules, I. K., Skene, A. M., Tan, M. H., Lefebvre, P. J., Murray, G. D. et al. (2005) Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* **366**, 1279–1289
- 182 Betteridge, D. J., DeFronzo, R. A. and Chilton, R. J. (2008) PROactive: time for a critical appraisal. *Eur. Heart J.* **29**, 969–983
- 183 Home, P. D., Pocock, S. J., Beck-Nielsen, H., Gomis, R., Hanefeld, M., Jones, N. P., Komajda, M. and McMurray, J. J. (2007) Rosiglitazone evaluated for cardiovascular outcomes: an interim analysis. *N. Engl. J. Med.* **357**, 28–38
- 184 Swedberg, K., Pfeffer, M., Granger, C., Held, P., McMurray, J., Ohlin, G., Olofsson, B., Ostergren, J. and Yusuf, S. (1999) Candesartan in heart failure: assessment of reduction in mortality and morbidity (CHARM): rationale and design. *Charm-Programme Investigators. J. Card. Failure* **5**, 276–282
- 185 Nystrom, T. (2008) The potential beneficial role of glucagon-like peptide-1 in endothelial dysfunction and heart failure associated with insulin resistance. *Horm. Metab. Res.* **40**, 593–606
- 186 Nikolaidis, L. A., Mankad, S., Sokos, G. G., Miske, G., Shah, A., Elahi, D. and Shannon, R. P. (2004) Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* **109**, 962–965
- 187 Sokos, G. G., Nikolaidis, L. A., Mankad, S., Elahi, D. and Shannon, R. P. (2006) Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J. Card. Failure* **12**, 694–699
- 188 Fragasso, G., Pallosi, A., Puccetti, P., Silipigni, C., Rossodivita, A., Pala, M., Calori, G., Alfieri, O. and Margonato, A. (2006) A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. *J. Am. Coll. Cardiol.* **48**, 992–998
- 189 Lee, L., Campbell, R., Scheuermann-Freestone, M., Taylor, R., Gunaruwan, P., Williams, L., Ashrafian, H., Horowitz, J., Fraser, A. G., Clarke, K. and Frenneaux, M. (2005) Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation* **112**, 3280–3288
- 190 McCormack, J. G., Barr, R. L., Wolff, A. A. and Lopaschuk, G. D. (1996) Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. *Circulation* **93**, 135–142
- 191 Patel, A., MacMahon, S., Chalmers, J., Neal, B., Woodward, M., Billot, L., Harrap, S., Poulter, N., Marre, M., Cooper, M. et al. (2007) Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* **370**, 829–840
- 192 Yusuf, S., Teo, K. K., Pogue, J., Dyal, L., Copland, I., Schumacher, H., Dagenais, G., Sleight, P. and Anderson, C. (2008) Telmisartan, ramipril, or both in patients at high risk for vascular events. *N. Engl. J. Med.* **358**, 1547–1559
- 193 Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R. and Dagenais, G. (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N. Engl. J. Med.* **342**, 145–153
- 194 Lewis, E. J., Hunsicker, L. G., Clarke, W. R., Berl, T., Pohl, M. A., Lewis, J. B., Ritz, E., Atkins, R. C., Rohde, R. and Raz, I. (2001) Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N. Engl. J. Med.* **345**, 851–860
- 195 Shekelle, P. G., Rich, M. W., Morton, S. C., Atkinson, C. S., Tu, W., Maglione, M., Rhodes, S., Barrett, M., Fonarow, G. C., Greenberg, B. et al. (2003) Efficacy of angiotensin-converting enzyme inhibitors and  $\beta$ -blockers in the management of left ventricular systolic dysfunction according to race, gender, and diabetic status: a meta-analysis of major clinical trials. *J. Am. Coll. Cardiol.* **41**, 1529–1538
- 196 Cohn, J. N. and Tognoni, G. (2001) A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. *N. Engl. J. Med.* **345**, 1667–1675
- 197 Wlodarczyk, J. H., Keogh, A., Smith, K. and McCosker, C. (2003) CHART: congestive cardiac failure in hospitals, an Australian review of treatment. *Heart Lung Circ.* **12**, 94–102
- 198 Bakris, G. L., Fonseca, V., Katholi, R. E., McGill, J. B., Messerli, F. H., Phillips, R. A., Raskin, P., Wright, Jr, J. T., Oakes, R., Lukas, M. A. et al. (2004) Metabolic effects of carvedilol vs metoprolol in patients with type 2 diabetes mellitus and hypertension: a randomized controlled trial. *JAMA, J. Am. Med. Assoc.* **292**, 2227–2236
- 199 (1999) The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet* **353**, 9–13
- 200 Packer, M., Coats, A. J., Fowler, M. B., Katus, H. A., Krum, H., Mohacsi, P., Rouleau, J. L., Tendra, M., Castaigne, A., Roecker, E. B. et al. (2001) Effect of carvedilol on survival in severe chronic heart failure. *N. Engl. J. Med.* **344**, 1651–1658
- 201 Nemoto, O., Kawaguchi, M., Yaoita, H., Miyake, K., Maehara, K. and Maruyama, Y. (2006) Left ventricular dysfunction and remodeling in streptozotocin-induced diabetic rats. *Circ. J.* **70**, 327–334

- 202 Fredersdorf, S., Thumann, C., Ulucan, C., Griese, D. P., Luchner, A., Riegger, G. A., Kromer, E. P. and Weil, J. (2004) Myocardial hypertrophy and enhanced left ventricular contractility in Zucker diabetic fatty rats. *Cardiovasc. Pathol.* **13**, 11–19
- 203 Poornima, I. G., Parikh, P. and Shannon, R. P. (2006) Diabetic cardiomyopathy: the search for a unifying hypothesis. *Circ. Res.* **98**, 596–605
- 204 Barouch, L. A., Berkowitz, D. E., Harrison, R. W., O'Donnell, C. P. and Hare, J. M. (2003) Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation* **108**, 754–759
- 205 Rutter, M. K., Parise, H., Benjamin, E. J., Levy, D., Larson, M. G., Meigs, J. B., Nesto, R. W., Wilson, P. W. and Vasan, R. S. (2003) Impact of glucose intolerance and insulin resistance on cardiac structure and function: sex-related differences in the Framingham Heart Study. *Circulation* **107**, 448–454
- 206 Tenenbaum, A., Fisman, E. Z., Schwammenthal, E., Adler, Y., Benderly, M., Motro, M. and Shemesh, J. (2003) Increased prevalence of left ventricular hypertrophy in hypertensive women with type 2 diabetes mellitus. *Cardiovasc. Diabetol.* **2**, 14
- 207 Yarom, R., Zirkin, H., Stammer, G. and Rose, A. G. (1992) Human coronary microvessels in diabetes and ischaemia. Morphometric study of autopsy material. *J. Pathol.* **166**, 265–270
- 208 Nunoda, S., Genda, A., Sugihara, N., Nakayama, A., Mizuno, S. and Takeda, R. (1985) Quantitative approach to the histopathology of the biopsied right ventricular myocardium in patients with diabetes mellitus. *Heart Vessels* **1**, 43–47
- 209 Kawaguchi, M., Asakura, T., Saito, F., Nemoto, O., Maehara, K., Miyake, K., Sugai, N. and Maruyama, Y. (1999) [Changes in diameter size and F-actin expression in the myocytes of patients with diabetes and streptozotocin-induced diabetes model rats]. *J. Cardiol.* **34**, 333–339

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