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Application of Animal Models in Diabetic Cardiomyopathy

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Diabetic heart disease is a growing and important public health risk. Apart from the risk of coronary artery disease or hypertension, diabetes mellitus (DM) is a well-known risk factor for heart failure in the form of diabetic cardiomyopathy (DiaCM). Currently, DiaCM is defined as myocardial dysfunction in patients with DM in the absence of coronary artery disease and hypertension. The underlying pathomechanism of DiaCM is partially understood, but accumulating evidence suggests that metabolic derangements, oxidative stress, increased myocardial fibrosis and hypertrophy, inflammation, enhanced apoptosis, impaired intracellular calcium handling, activation of the renin-angiotensin-aldosterone system, mitochondrial dysfunction, and dysregulation of microRNAs, among other factors, are involved. Numerous animal models have been used to investigate the pathomechanisms of DiaCM. Despite some limitations, animal models for DiaCM have greatly advanced our understanding of pathomechanisms and have helped in the development of successful disease management strategies. In this review, we summarize the current pathomechanisms of DiaCM and provide animal models for DiaCM according to its pathomechanisms, which may contribute to broadening our understanding of the underlying mechanisms and facilitating the identification of possible new therapeutic targets.

Keywords: Cardiomyopathies; Diabetes mellitus; Disease models, animal; Heart failure

INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing at a critical rate; recent assumptions predict that 642 million adults worldwide will be affected by DM by 2040 [1,2]. Importantly, diabetic patients have an increased risk of chronic complications, including retinopathy, neuropathy, nephropathy, and cardiovascular disease [1,3,4].

The Framingham Heart Study revealed that the risk of heart failure (HF) increases 2- to 8-fold in the presence of type 2 diabetes mellitus (T2DM) and that 19% of patients with HF have T2DM [5,6]. In fact, patients with diabetes can develop a unique form of HF, termed diabetic cardiomyopathy (DiaCM), which is characterized by initial diastolic dysfunction without systolic dysfunction, often referred to as HF with preserved ejection fraction (HFpEF), eventually progressing to HF with reduced ejection fraction [7,8]. DM elicits changes in several cell types in the heart, including cardiac fibroblasts, endothelial cells, cardiomyocytes, and inflammatory cells. These changes promote detrimental cardiac remodeling, including cardiac fibrosis, cardiomyocyte apoptosis, and myocardial hypertrophy [1,9,10].

Many animal models of chronic hyperglycemia exist, each replicating certain aspects of clinical DM. These animal models use genetic engineering, obesogenic diets and pancreatic toxins to induce DM. In terms of DiaCM, several models of DM have been shown to cause diastolic dysfunction [1]. Despite these efforts, effective treatment options have remained

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elusive, partly due to the limitations of an experimental model that adequately mimics human DiaCM [1].

This review provides an overview of the pathomechanisms of DiaCM. We also describe the small animal models for DiaCM according to its pathomechanisms. These findings will aid our understanding of the pathophysiology of DiaCM and hopefully advance the discovery of new therapeutic strategies for this unique disease entity.

PATHOGENESIS OF DIABETIC CARDIOMYOPATHY

The pathomechanisms underlying the development of DiaCM are multifactorial and incompletely understood. There are various proposed mechanisms of DiaCM, including metabolic disturbances, insulin resistance, cardiac autonomic dysfunction, maladaptive immune responses, subcellular component

Table 1. Animal models for type 1 and type 2 diabetes mellitus

Model	Species	Intervention	Manipulation	Target	DM onset	Phenotypes
Type 1 DM						
STZ [14]	Mice	Pharmacological	Injection	β-Cell	2 day	Necrosis & loss of insulin production, hyperlipidemia
Alloxan [20]	Mice	Pharmacological	Injection	β-Cell	5 day	Necrosis & loss of insulin production, high TG
OVE26 [21]	Mice	Transgenic	Overexpression	Calmodulin	2–3 wk	β-cell damage, high TG
NOD [17]	Mice	Transgenic	Insulitis	β-Cell	30 wk	β-cell failure, high TG
Akita [14,17]	Mice	Transgenic	Spontaneous missense mutation	Insulin-2 gene	5–6 wk	Misfolding of insulin protein, facilitate ER stress, β-cell failure, high TG
Type 2 DM						
HFD/HSD [14,21]	Mice	Diet-induced	Feeding		1 wk	Obesity, high TG
HFD+low dose STZ [14,21]	Mice	Diet & Pharmacological	Feeding+injection	β-Cell	2–10 wk	Obesity, IR
ob/ob [14,21]	Mice	Transgenic	Deficiency	Leptin	8–15 wk	Obesity, IR, high TG, FFA
db/db [14,21]	Mice	Transgenic	Nonfunctioning	Leptin receptor	4–8 wk	Obesity, IR, high TG, FFA
ZF/ZDF [14,21]	Rats	Transgenic	Nonfunctioning	Leptin receptor	14 wk	Obesity, high TG
GK [18,20]	Rats	Transgenic	Overexpression	SREBP-1c	3 wk	IR, high TG, FFA
OLETF [20,21]	Rats	Polygenic	Food-intake control defect	CCK-1R, Odb2	18 wk	Obesity, high TG
KK-Ay [17,20]	Mice	Polygenic	Spontaneous	Agouti gene	8–16 wk	Obesity, high TG, IR
NZO/HiLt (male) [17,18]	Mice	Polygenic	Spontaneous	Ab to leptin transporter	12–24 wk	Obesity, leptin resistance, IR
TallyHo/JngJ (male) [17,18]	Mice	Polygenic	Spontaneous	Tanidd1-3	10–16 wk	Obesity, hyperlipidemia, hyperinsulinemia
NONcNZO10/LtJ [18,20]	Mice	Polygenic	Spontaneous	Zinc homeostasis or glucose metabolism	8–24 wk	Obesity, IR

DM, diabetes mellitus; STZ, streptozotocin-induced mice; TG, triglyceride; OVE26, OVE26 diabetic mice; NOD, nonobese diabetic mice; Akita, a C57BL/6NSlc mouse with a spontaneous mutation in the insulin-2 gene; ER, endoplasmic reticulum; HFD/HSD, high-fat/high-sucrose diet; IR, insulin resistance; *ob/ob*, leptin-deficient mice; FFA, free fatty acid; *db/db*, leptin receptor-deficient mice; ZF, Zucker fatty rats; ZDF, Zucker diabetic fatty rats; GK, Goto-Kakizaki rats; CCK-1R, cholecystokinnin-1 receptor; Odb2, diabetogenic gene located on chromosome 14; SREBP-1c, sterol regulatory element-binding protein-1c; OLETF, Otsuka Long-Evans Tokushima fatty rats; KK-Ay, yellow obese gene transgenic Kuo Kondo mice; NZO, New Zealand obese mice; Ab, antibody; Tanidd1, a mouse chromosome 19 quantitative trait loci associated with diabetes in TALLYHO mice; NONcNZO10/LtJ, a recombinant congenic strain comprising approximately 88% genome contribution from the NON/LtJ (nonobese and nondiabetic) strain and 12% from the New Zealand obese strain.

abnormalities, microvascular impairment, and alterations in the renin-angiotensin-aldosterone system (RAAS) [5,11,12]. These factors induce the activation of multiple inflammatory pathways and increase oxidative stress, which mediate extracellular and cellular injuries, thus ultimately inducing pathological cardiac remodeling [5,13].

ANIMAL MODELS ACCORDING TO PATHOMECHANISMS OF DIABETIC CARDIOMYOPATHY

Rodents, especially rats and mice, are powerful tools to investigate the pathophysiological mechanisms involved in the development of DiaCM. Rat or mouse genomes are approximately the same size as the human genome, each containing nearly 30,000 protein-coding genes, with approximately 99% of the genes encoded in the mouse genome having a homologue in humans [14-16]. In addition to these genomic resemblances, further benefits of mouse models include the short breeding cycle and the usefulness of a variety of genetically engineered loss- and gain-of-function models [14,17]. The commonly used rodent models to produce type 1 diabetes mellitus (T1DM) and T2DM are summarized in Table 1 [14,17-21] and Fig. 1. The following sections will describe the animal models according to the pathomechanisms of DiaCM observed in T1DM and T2DM.

Metabolic derangements

Innumerable studies apply dietary manipulations to induce obesity, insulin resistance, and T2DM in rodents and large animal models [14,17,19]. Insulin signaling in the heart is preserved in T2DM rodent models following short-term high-fat diet (HFD) feeding [22,23]. However, prolonged HFD feeding in animal models impairs its downstream targets of the serine/threonine kinase Akt and forkhead box O-1 (FOXO1) transcription factor phosphorylation [24], which results in persistent FOXO1 nuclear localization and activation. Mice with cardiac-specific deletion of glucose transporter type 4 (GLUT4)

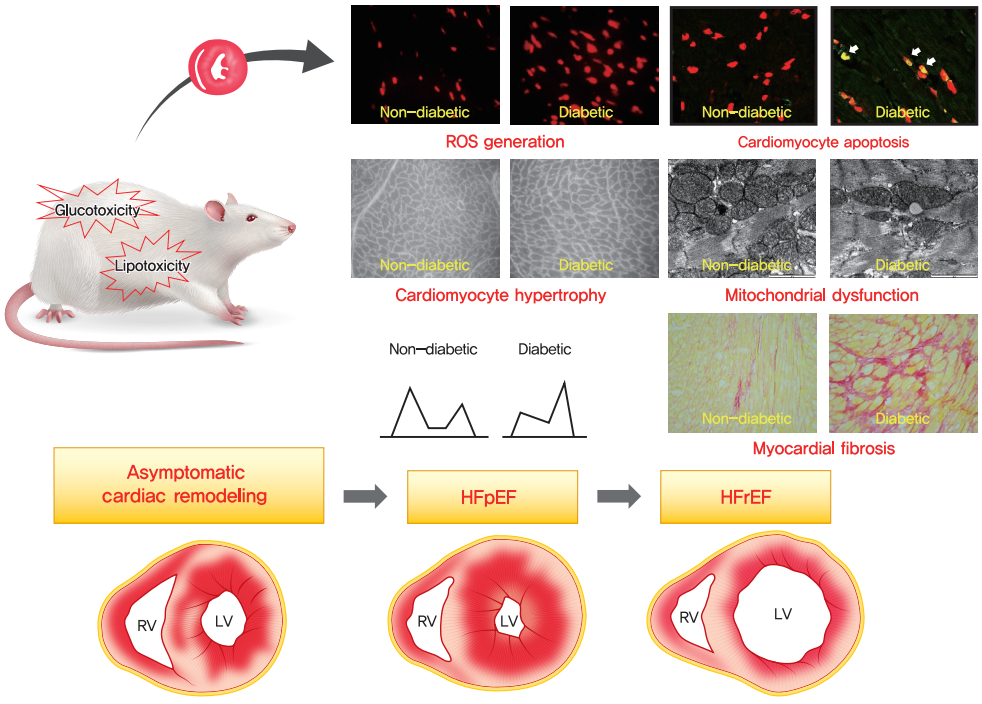

Fig. 1. Pathological and functional changes of diabetic cardiomyopathy. The pathologies of the diabetic hearts show that the increases in reactive oxygen species generation, apoptosis, cardiac hypertrophy, mitochondrial dysfunction, and myocardial fibrosis than non-diabetic heart. Diabetes mellitus (vs. no diabetes mellitus) is also associated with heart failure with preserved ejection fraction characterized by reduced compliance (reduced mitral E/A ratio) and diastolic dysfunction. ROS, reactive oxygen species; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; RV, right ventricle; LV, left ventricle.

showed normal cardiac function in the unstressed state but developed maladaptive hypertrophy and severe contractile dysfunction in response to left ventricular (LV) pressure overload [13,25]. Therefore, GLUT4 is required for the maintenance of cardiac function and structure in response to pathological processes that increase energy demand, in part through secondary changes in mitochondrial metabolism and cellular stress survival signaling, such as the phosphoinositide 3-kinase (PI3K)–Akt pathway [13,25].

In addition to stimulating glucose uptake, both insulin signaling [13,26] and cardiomyocyte contraction [13,27] can promote fatty acid uptake into cardiomyocytes via induction of cluster of differentiation 36 (CD36) translocation to sarcolemma membranes [26,28]. The long-lasting presence of CD36 at the sarcolemma membrane leads to an increased rate of long-chain fatty acid uptake and accumulation of triglycerides in cardiomyocytes, which results in lipotoxic DiaCM [28,29].

The transcription factor peroxisome proliferator-activated receptor-α (PPARα) is a major regulator of lipid metabolism and can increase the expression of genes encoding CD36, fatty acid-binding proteins and proteins involved in β-oxidation in the mitochondria and peroxisome [13,30]. Tribbles-related protein 3 (TRB3) can directly bind to Akt and inhibit Akt phosphorylation [13,31,32]. The expression of TRB3 is upregulated in the heart in T1DM and T2DM rodent models [33,34] and in skeletal muscle in patients with T2DM [32]. Furthermore, a rat model of T2DM induced by a HFD and low-dose streptozotocin (STZ) demonstrated severe insulin resistance and properties of DiaCM, including myocardial fibrosis, cardiac inflammation and LV dysfunction, in addition to increased expression of TRB3, compared with control rats [34].

The hearts from rats with T2DM infused *ex vivo* with the CD36 inhibitor sulfo-N-succinimidyl oleate (SSO) before inducing hypoxia, which resulted in a 29% reduction in the rate of fatty acid oxidation and an approximately 50% reduction in triglyceride concentration compared with vehicle treatment, showed a restoration of fatty acid metabolism to control levels following hypoxia–reoxygenation [13,35]. SSO infusion into diabetic rat hearts *ex vivo* before hypoxia also prevented cardiac dysfunction [35]. Fenofibrate treatment prevented fibrosis and diastolic dysfunction in diabetic rats, probably through improvements in cardiac and systemic lipid metabolism [36,37]. Fenofibrate treatment was also associated with reductions in markers of apoptosis and cardiac hypertrophy in rats with STZ-induced T1DM [38]. The glucagon-like peptide-1 (GLP1) analog liraglutide protected against the development of DiaCM in a rat model of STZ-induced T1DM by inhibiting the endoplasmic reticulum (ER) stress pathway [39]. Similarly, the GLP1 analog exendin-4 prevented the development of DiaCM via the amelioration of lipotoxicity in a mouse model of T2DM [40]. The dipeptidyl peptidase-4 (DPP4) inhibitor sitagliptin reduced blood glucose levels, increased GLP1 levels and prevented T2DM-induced DiaCM in mice by shifting the energy substrate utilization in the heart from fatty acids towards glucose [41,42]. Recently, sodium-glucose cotransporter type 2 (SGLT-2) inhibitors, novel hypoglycemic agents that increase urinary Na^+ and glucose excretion, were introduced to DM and DiaCM research and have come into the spotlight. In addition to the beneficial effects of SGLT-2 inhibitors on glucose-lowering or natriuretic action, several potential cardioprotective mechanisms of SGLT-2 inhibitors have been reported [5,13,43]. A number of studies have shown the multiple effects of SGLT-2 inhibitors on cardiac iron homeostasis, antioxidative stress, anti-inflammation, RAAS activity, antifibrosis, and GlcNAcylation, as well as mitochondrial function in the heart [43-47]. Excessive O-GlcNAcylation following chronic activation of the hexosamine biosynthetic pathway is associated with posttranslational modifications in the diabetic heart. O-GlcNAcylation impairs cardiac mitochondrial function, Ca^{2+} homeostasis, and ER stress in DM. A previous study showed that dapagliflozin prevented DiaCM by reducing the levels of O-GlcNAcylated protein in diabetic mice. These results demonstrated that O-GlcNAcylated levels of FOXO1 reduced by SGLT-2 inhibitors contributed to attenuation of DiaCM and improvement in heart function [43,46].

Oxidative stress

Excess generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) is considered to be a central mechanism for diabetes-associated inflammation and remodeling in the heart [13,48,49] and contributes to oxidative stress during both the early and late stages of DiaCM [50,51]. Defects in the antioxidant defense system further increase oxidative stress during the later stages of DiaCM [50,51]. Superoxide dismutase (SOD) has an important role in preventing cardiac damage in the setting of DM. Injection of the SOD mimic mitochondria-targeted mitochondrial triphenylphosphonium chloride (mito-TEMPO) prevented the hyperglycemia-induced increase in superoxide generation, reduced myocardial hypertrophy and improved myocardial function in STZ-in-

duced T1DM mice and *db/db* T2DM mice compared with vehicle treatment [52].

The transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) is an essential regulator of the antioxidant response with an important role in preventing diabetes-induced oxidative stress and cell death. Isolated cardiomyocytes from Nrf2 knockout (KO) mice were more susceptible to high glucose-induced cell death than wild-type (WT) cells [13,53]. Furthermore, NRF2-deficient mice were more susceptible to diabetes-induced or angiotensin (Ang) II-induced cardiomyopathy than WT mice, whereas cardiomyocyte-specific overexpression of Nrf2 conferred resistance to Ang II-induced cardiomyopathy [54,55]. Naturally occurring activators of NRF2 have been shown to ameliorate diabetes-induced cardiac complications. Sulforaphane is an organosulfur compound derived from cruciferous vegetables such as cabbage, Brussels sprouts, and broccoli that has been shown to upregulate the expression of numerous genes encoding antioxidant proteins by activating NRF2 signaling [13,56]. The cardioprotective benefits of sulforaphane in attenuating fibrosis, oxidative damage, inflammation, hypertrophy, and cardiac dysfunction have been demonstrated in both T1DM and T2DM mouse models and in mice exposed to Ang II [54,55,57,58]. Administration of the antioxidant N-acetylcysteine (NAC) for 5 weeks to rat and mouse models of STZ-induced T1DM normalized the levels of oxidative stress and subsequently prevented the development of DiaCM [59,60]. Interestingly, the earlier the NAC treatment protocol was initiated after induction of diabetes with STZ during the 12-week experiment, the greater the protection against DiaCM [60], suggesting that early damage mediated by increased oxidative stress has a more important role in the development of DiaCM. In diabetic rats, NAC treatment attenuated cardiac dysfunction and damage after myocardial ischemia–reperfusion injury [61,62].

Myocardial fibrosis and hypertrophy

Systemic inflammation, hyperglycemia, and dyslipidemia associated with DM lead to the development of cardiac fibrosis and hypertrophy, which increase myocardial stiffness and result in LV diastolic and systolic dysfunction [13].

In DiaCM, increased collagen accumulation is observed in perivascular loci, intermyofiber spaces, and replacement fibrosis [14]. Thus, cardiac fibrosis increased in some animal models of both T1DM [14,63-66] and T2DM [67,68]. Under diabetic conditions, advanced glycation end products created by the exposure of proteins and lipids to high glucose levels crosslink extracellular matrix (ECM) proteins, impair ECM degradation by matrix metalloproteinases and increase cardiac stiffness, which together manifest as early LV diastolic dysfunction [13,69,70]. Genetically obese mice exhibited severe diastolic dysfunction, as evidenced by decreasing the ratio of the early (E) to late (A) (E/A) velocities in *db/db* and *ob/ob* mice [21,71,72]. Contractile properties were still slightly affected in *ob/ob* mice [75], while *db/db* mice displayed reduced fractional shortening and velocity of circumferential fiber shortening at 12 weeks of age [21,72].

Epicardial and endothelial cells can also contribute to the development of cardiac fibrosis through epithelial-to-mesenchymal or endothelial-to-mesenchymal transition to myofibroblasts [13,73-75].

The antifibrotic agent cinnamoyl anthranilate reduced collagen production stimulated by transforming growth factor β (TGF-β) signaling in cultured renal mesangial cells [76]. Administration of FT23 and FT011, which are derivatives of cinnamoyl anthranilate, attenuated cardiac structural and functional abnormalities in an animal model of DiaCM [77,78].

Inflammation and cytokines

In the diabetic heart, chemokines, cytokines, and exosomes secreted by inflammatory cells contribute to the development of cardiomyocyte hypertrophy and ECM remodeling. Several myocardial processes are activated by a number of proinflammatory factors, dyslipidemia, hyperglycemia, and elevated Ang II levels that are upregulated in the setting of DM [13]. Together, these factors promote the infiltration and accumulation of proinflammatory lymphocytes and macrophages into the lesion site. These inflammatory cells secrete cytokines such as TGF-β, interleukin (IL)-1β, tumor necrosis factor (TNF), IL-6, and interferon-γ that can cause or exacerbate myocardial injury, contributing to further adverse cardiac remodeling [79,80].

Mice with STZ-induced T1DM have higher T cell infiltration into the myocardium, which is associated with increased myocardial fibrosis and LV dysfunction, than control mice [81]. Inhibition of T cell trafficking in diabetic mice prevented myocardial fibrosis and cardiac dysfunction [82,83].

Toll-like receptor 4 (TLR4) is expressed in cardiomyocytes, inflammatory cells, and cardiac fibroblasts in both normal and failing hearts [13]. The role of TLR4-mediated inflammatory signaling in the development of DiaCM has been reported in animal models of T1DM and T2DM [84,85]. Inflammatory

factors, including nuclear factor-κB and TNF, and protein kinases, such as c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), can directly lead to cardiomyocyte hypertrophy and can advance myocardial fibrosis [86,87]. Activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, a regulator of cell death and inflammation [88], has been associated with cardiac inflammation, fibrosis, and cell death triggered by HFD and STZ administration in a rat model of T2DM [89]. These effects were attenuated by microRNA (miRNA)-mediated Nlrp3 silencing [89] or by pharmacological suppression of NLRP3 inflammasome activation [90].

Suppression of TLR4 signaling with triptolide or matrine improved cardiac LV function and reduced collagen accumulation in rat models of DiaCM [91,92]. Long-term blockade of TLR4 with the TLR4 inhibitor TAK-242 (also known as CLI-095) was associated with a slight improvement in diabetes-induced erectile dysfunction in rats compared with no treatment, mediated by an increase in cyclic guanosine monophosphate levels and the attenuation of oxidative stress in penile tissue [93]. Numerous small-molecule inhibitors of the NLRP3 inflammasome have evolved in the past several years. The orally active NLRP3 inhibitor 16673-34-0 prevented Western diet-induced systolic and diastolic LV dysfunction in obese mice [94].

Cardiomyocyte damage and apoptosis

Apoptosis is an extremely controlled mechanism of programmed cell death and seems to be the principal form of cell death in DiaCM, compared with lower rates due to necrosis [95,96].

In T1DM animals, both increased death receptor signaling and mitochondria-dependent proapoptotic signaling led to elevated apoptosis in DiaCM, and antioxidant treatment diminished both of these signaling pathways and apoptosis, suggesting an essential role of increased ROS in apoptosis induction in DiaCM [95,97]. A recent study also proposed that dissociation of B-cell lymphoma 2 (Bcl-2) protein from beclin-1 by restoration of impaired AMP-dependent protein kinase (AMPK) activity may decrease apoptosis in DiaCM by restoring autophagy, supporting the suggestion that an interplay between apoptosis and autophagy may be important in DiaCM [98]. Furthermore, ER stress may encourage apoptosis in DiaCM by activating JNK signaling and apoptosis via the extrinsic and intrinsic pathways or by increasing protein kinase RNA-like ER kinase (PERK)-C/EBP homologous protein (CHOP) signaling, which may provoke apoptosis by switching expression towards proapoptotic Bcl-2 proteins [99].

Impaired CA^{2+} handling

In DM, the process of cardiac calcium cycling (Ca^{2+} entry, intracellular Ca^{2+} concentration, and Ca^{2+} efflux) is modified in both humans and animal models, contributing to impaired cardiac contraction and relaxation. Decreased Ca^{2+} entry is the consequence of both altered voltage dependence of the L-type calcium channel (LTCC) and reduced expression [95]. Impaired intracellular Ca^{2+} cycling consists of reductions in the amplitude of Ca^{2+} and in the systolic rate of the Ca^{2+} rise and fall [100,101]. Prolonged rates of Ca^{2+} decay may arise from impaired sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) activity during the diastolic period, which may cause a decrease in sarcoplasmic reticulum (SR) Ca^{2+} storage of up to 50% and, thus, can lead to diastolic dysfunction and impaired relaxation [102].

In models of T2DM, contractile dysfunction may be driven by a significant decrease in the Ca^{2+} transient due to reduced Ca^{2+} influx as a consequence of decreased LTCC expression, by decreased SR Ca^{2+} content due to increased phospholamban expression and decreased SERCA2a expression, and by the diminished activity and content of ryanodine receptor (RyR) [95,103]. In addition, hyperglycemia may lead to the O-GlcNAcylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which may accelerate diastolic SR Ca^{2+} leakage via RyRs, leading to SR Ca^{2+} depletion [104].

A potent late Na^+ current inhibitor, ranolazine, might normalize altered intracellular Ca^{2+} levels in cardiomyocytes due to the close relationship between Ca^{2+} and Na^+ coupling handled by the Na^+/Ca^{2+} exchanger [5,105]. Ranolazine improved several hemodynamic parameters but not cardiac relaxation variables. This result showed that a single treatment using ranolazine is probably not sufficient to influence myocardial structure and cardiac function [5,105].

Renin-angiotensin-aldosterone system activation

Current evidence from animal experiments and human patients has identified a critical role for RAAS in DiaCM [5]. Cytoplasmic Ang II enhances cell growth in animal models. Ang II has a definite influence on cell signaling, resulting in cardiomyocyte hypertrophy and proliferation of cardiac fibroblasts [106]. Other factors, such as inflammation, oxidative stress,

and aldosterone, may potentiate the harmful effects of Ang II on the heart that lead to myocardial damage in DM [107]. Moreover, the enhanced activation of Ang II and mineralocorticoid receptor signaling might promote insulin resistance by initiating the mammalian target of rapamycin (mTOR)-S6 kinase 1 signal transduction pathway [5,108].

Recently, renin inhibitors (aliskiren), angiotensin II receptor blockers (ARBs), and angiotensin converting enzyme inhibitors (ACEis) were shown to be protective medications against DiaCM in rat models [5,109]. ACEis and ARBs were also useful agents in both human and animal models of DiaCM [110, 111]. The favorable effect of β-adrenoreceptor blockers was also demonstrated in experimental models of DiaCM [112].

Mitochondrial dysfunction

Mitochondrial dysfunction is a well-known feature of DiaCM in both animal and human DM. Mitochondrial dysfunction refers to abnormal mitochondrial ultrastructure, increased mitochondrial oxidative stress, impaired activity of Ca^{2+}-sensitive dehydrogenases and F0F1-ATPase, increased sensitivity for Ca^{2+}-induced opening of the mitochondrial permeability transition pore, transcriptional and translational downregulation of oxidative phosphorylation (OXPHOS) subunits, and impaired mitochondrial respiratory capacity and coupling [95,113].

In humans, several studies have demonstrated mitochondrial dysfunction in the atrium and atrial appendages of DM patients [114-116], with impaired respiration rates and electron transport chain complex activities in patients with DM. In a diabetic mouse model, as early as 1985, an impairment in state 3 respiration of isolated cardiac mitochondria was observed [117]. Since then, mitochondrial dysfunctions have been reported in numerous diabetic rodent models [118]. In terms of T1DM models, STZ-treated rats showed reduced antioxidant glutathione, increased ROS production, and ultimately loss of mitochondrial membrane potential [119]. OVE26 mice also displayed a reduction in glutathione, altered mitochondrial function, and an increase in mitochondrial biogenesis [120]. Akita mice revealed an increased volume of mitochondria with reduced crista densities and respiratory defects [121]. In T2DM models, *db/db* mice displayed increases in O^2 consumption, lipid peroxidation, and mitochondrial ROS generation [122]. Otsuka Long-Evans Tokushima fatty (OLETF) and *ob/ob* mouse models maintained unchanged levels of uncoupled proteins despite mitochondrial dysfunction [123,124]. Zucker diabetic fatty (ZDF) rats showed increased lipid peroxidation and mitochondrial ROS production rates with elevated antioxidant levels [125,126]. Goto-Kakizaki (GK) and OLETF rats also revealed higher lipid peroxidation and mitochondrial ROS production [125,127,128]. The activity of Sirtuin 3 (SIRT3), a major regulator of intramitochondrial protein acetylation and NAD^+-dependent mitochondrial deacetylase, may be reduced in the diabetic heart, causing ROS deposition due to increased acetylation and, thus, suppression of manganese superoxide dismutase (MnSOD) [95,129]. Furthermore, SIRT3 deficiency seems to exacerbate suppression of mitophagy and autophagy in the diabetic heart, whereas SIRT3 overexpression promoted mitophagy and autophagy, attenuated cardiomyocyte apoptosis and diminished mitochondrial defects [130].

MicroRNAs

In DiaCM, dysregulations of 316 out of 1,008 total miRNAs were discovered, and pathway analysis demonstrated that several miRNAs are involved in cardiac hypertrophy, oxidative stress, apoptosis, and autophagy [95,131].

Adenovirus-mediated rescue of the myocardial proviral integration site for Moloney murine leukemia virus-1 (Pim-1) expression *in vivo* improved systolic and diastolic function, attenuated apoptosis and fibrosis, attenuated ventricular dilation, and restored SERCA2a content in DiaCM [132,133]. The expression of miR-133 is decreased in the STZ-induced diabetic mice, and miR-133 has direct inhibitory effects on collagen deposition by deteriorating connective tissue growth factor expression, indicating that increased miR-133 concentrations may attenuate myocardial fibrosis in DiaCM [134]. Myocardial expression of miR-451 is distinctly increased in mice fed a HFD, and cardiomyocyte-specific deletion of miR-451 decreases ceramide deposition, cardiac hypertrophy, and myocardial fibrosis in this mouse model. Diminution of hypertrophy may come from restoration of attenuated AMPK activity, which may normalize increased mTOR phosphorylation and thus restrict HFD-induced cardiomyocyte growth [135]. Upregulation of miR-30d in DiaCM was suggested to reduce FoxO3a signaling, causing caspase 1 activation and increasing inflammatory signaling, thus resulting in pyroptosis [136]. Based on the various characteristics and mechanisms of DiaCM that can be controlled by miRNAs, a significant contribution of miRNAs to the development of DiaCM was suggested [137].

ANIMAL MODELS FOR HEART FAILURE WITH PRESERVED EJECTION FRACTION

Clinically, diastolic HF and HFpEF are not synonymous [138,139]. HFpEF is a clinical term used to imply HF with normal systolic function but without any consideration of diastolic function [140,141]. Although diastolic HF and HFpEF are not synonymous, many clinical features overlap [140-142]. Chronic prolonged diastolic dysfunction is a clear risk factor for HFpEF [140,141]. For this reason, an experimental model of human HFpEF generally requires evaluations of ventricular hypertrophy, diastolic dysfunction, exercise intolerance, and interstitial fibrosis [143]. Several established rodent models of HFpEF are summarized in Table 2 [138,144-149].

A rodent model of HFpEF represents cardiac stiffness and hypertrophy with interstitial fibrosis [143]. Hence, the measurement of LV wall thickness is obligatory to indicate hypertrophy. A number of studies have used LV free wall thickness to demonstrate hypertrophy. Moreover, myocardial interstitial fibrosis and perivascular fibrosis are usually present with hypertrophy [150]. Because a major component of myocardial fibrosis is the presence of collagen in the ECM, collagen staining can manifest its severity [138,151].

Since DM and obesity are notable comorbidities in HFpEF [152], genetically modified *db/db* [153] or *ob/ob* [154] mice are widely applied for cardiometabolic explorations. Diastolic dysfunction of LV has been described for both models [71,155,156]. Additional rodent models for insulin resistance and T2DM include Zucker fatty (ZF) rats, which represent nonfunctional leptin receptors [157], and ZDF rats, which are further inbred strains of ZF rats with high serum glucose concentrations [158]. Recently, Schiattarella et al. [159] created a nongenetic and noninvasive modified model of HFpEF that combined hypertension and hyperlipidemia. They administered a HFD with a nitric oxide synthase inhibitor ad libitum. After 5 weeks, they verified significant impairment of diastolic function, with exercise intolerance and pulmonary congestion. At 15 weeks, significant symptoms and signs of HFpEF had developed [159]. This novel model mimics human pathophysiology, suggesting its appropriateness for use in future research [138].

THE DIFFERENCES BETWEEN HUMAN PATIENTS AND ANIMAL MODELS IN DIABETIC CARDIOMYOPATHY

Innumerable small animal models have been created to explore the impacts of T1DM and T2DM on the heart [14,155]. However, animal models have some limitations and differences

Table 2. Rodent models for heart failure with preserved ejection fraction

Strain	Model	Manipulation	Phenotypes
Mice			
ob/ob	Obesity [138,153]		Hypertrophy, diastolic dysfunction
db/db	DM [138,154]		IR, hypertrophy, diastolic dysfunction
SAMP8	Aging [144]		Diastolic dysfunction
C57BL/6	Obesity+HTN [145]	HFD+L-NAME	Hypertrophy, diastolic dysfunction, pulmonary congestion
	HTN [146]	TAC	Hypertrophy, fibrosis, diastolic dysfunction
	HTN [138,147]	Aldosterone, unilateral nephrectomy, 1% NaCl water-drinking	Hypertrophy, fibrosis, diastolic dysfunction
	HTN [145,148]	DOCA salt, unilateral nephrectomy, 1% NaCl water-drinking	Mild HTN, hypertrophy
Rats			
Wistar	HTN [149]	DOCA salt, unilateral nephrectomy, 1% NaCl water-drinking	Severe HTN, hypertrophy
DSS	HTN [138,148]	4%–8% NaCl chow	Severe HTN, diastolic HF

ob/ob, mice with leptin deficiency; *db/db*, mice with leptin receptor deficiency; DM, diabetes mellitus; IR, insulin resistance; SAMP8, mice with senescence-accelerated mouse prone 8; HTN, hypertension; HFD, high-fat diet; L-NAME, L-NG-nitroarginine methyl ester; TAC transverse aortic constriction; DOCA, deoxycorticosterone acetate; DSS, Dahl salt-sensitive rats; HF, heart failure.

from human patients. First, in general, rodents have very similar or identical genetic backgrounds, which is the main limitation, as the models do not reflect human genetic heterogeneity [17,20]. The second limitation of animal models is the rapid induction of stress factors, which is in contrast to the generally slow progression of disease in the human population [17,155]. Third, of several differences between murine and human hearts, heart rate is the fundamental difference between them. On the basis of these contractile kinetics, the ability to increase heart rates in small animal models is impaired compared with humans, which can usually increase by up to nearly threefold. Conversely, the heart rate of mice can increase by approximately 30% to 40% under exercise conditions, which restricts cardiac reserve and is a crucial consideration in the design of animal experiments [155].

Fourth, the STZ-induced diabetic model can reproduce most characteristics of human DiaCM associated with T1DM. However, STZ may also cause damage to nonpancreatic tissues such as the brain, and the accentuated and rapid onset of T1DM can differ from the disease onset in humans [17,21]. In relation to current data, the most appropriate T1DM model produced by chemical induction of pancreatic toxicity is achieved by STZ. However, the STZ-induced model is not a mutation-induced model. Therefore, it is not an appropriate rodent model to investigate glucose-related gene research, such as glucokinase and unique mutations in glucose-related genes [20,21]. In addition, animal models developing T1DM via point mutations, such as the OVE26 mouse model, also have fundamental limitations in terms of different mechanisms from human T1DM caused by autoimmune failure [21]. The lack of insulin production in the Akita model causes some unusual responses in the heart, such as the absence of fibrosis and hypertrophy and constant levels of oxidation despite mitochondrial dysfunction [20,21]. The GK rat model develops hyperglycemia, insulin resistance, and dyslipidemia with cardiac dysfunction, but obesity and steatosis, commonly observed in clinical practice, are not displayed well [19-21]. Consequently, considering the various limitations mentioned above, an ideal T1DM and T2DM rodent model should be generated in the future.

SUMMARY AND PERSPECTIVES

Animal models for DM, especially rats and mice, replicate many aspects of the pathogenesis of DiaCM and help to interpret potential contributing mechanisms of the disease [17,155]. A number of experimental protocols have been created to induce DM using supplemented diets, genetics and chemical-induced models [1,17,20]. STZ, a pancreatic β-cell toxin, is commonly used to induce β-cell necrosis and subsequent insulin deficiency [1,10,160]. Although this model does not replicate the clinically more prevalent T2DM, the STZ model evades confounding factors such as obesity and insulin resistance, which need to be taken into consideration in the common genetic models of T2DM, including spontaneous mutation of diabetic *db/db* and *ob/ob* mice [1,17]. Recently, there has been a development of T2DM models incorporating low-dose STZ with dietary intervention, as HFD alone is not enough to induce DM [17,20,161]. Remarkably, the use of low-dose STZ and HFD in a rat model mimics late-stage T2DM, when pancreatic β-cell dysfunction becomes obvious [162]. Another model of T1DM is the OVE26 mouse, which overexpresses calmodulin (a multifunctional Ca^{2+}-binding messenger protein) in pancreatic β-cells, resulting in pancreatic β-cell injury [14]. T1DM Akita mice ($Ins2Akita^{+/-}$) exhibit a spontaneous mutation in the insulin 2 gene, which promotes misfolding of the insulin protein, ER stress, and ultimately β-cell failure [20, 163].

Commonly used composite transgenic models for obesity, insulin resistance, and T2DM are *db/db* [153] or *ob/ob* [154] mice, which are based on leptin resistance or deficiency, respectively. ZF rats manifest obesity as a consequence of nonfunctional leptin receptors [164]. ZDF rats were produced by multiple further rounds of inbreeding ZF rats with high serum glucose levels [158]. GK rats are a spontaneous model of T2DM without obesity and are a selective inbred strain derived from Wistar rats [20,165]. Transgenic rodent models that mimic aspects of DiaCM have been created. For example, mice with adipose tissue-specific overexpression of sterol regulatory element-binding protein-1c (SREBP-1c) exhibit elevated plasma triglyceride levels and insulin resistance [14,166]. In addition, mice with cardiomyocyte-specific overexpression of the transcription factor PPARα driven by the α myosin heavy chain gene promoter (MHC-PPARα) display an increase in cardiac fatty acid oxidation and a similar phenotype as DiaCM [167]. Cardiomyocyte-selective insulin receptor KO (CIRKO) mice were used to investigate the effect of decreased insulin signaling in cardiomyocytes without causing systemic metabolic disturbances [168].

For T2DM-linked DiaCM, since human T2DM usually oc-

curs from unknown polygenic mutations with/without an unhealthy lifestyle, HFD animal models can be closer to ideal T2DM models that develop point mutations in the leptin system or in lipid storage. Polygenetic mutations of obesity, such as OLETF rats and yellow obese gene transgenic Kuo Kondo (KK-Ay) mice, should be further investigated, and nonobese polygenetically mutated GK rats could promote the study of T2DM itself [17,20,21].

There are several limitations to animal model research; thus, it is necessary to carefully interpret the results from the studies conducted. Fundamentally, genetic, structural, and immune system differences exist between humans and animals; thus, there is a limit to accurately predicting clinical results through diabetic animal models. In particular, it should be considered that different species of animals have different sensitivities to environmental factors, which may result in different symptoms and mechanisms of DiaCM. Despite these specific limitations, animal models, especially rats and mice, serve as inestimable creatures that have greatly developed our understanding of the pathogenesis of DiaCM or HF. Based on recent progress in genome editing, it is highly likely that innumerable novel transgenic models will be created in the near future [155].

To date, no agreement has been reached on the appropriate management strategy to prevent or treat cardiovascular complications associated with DM. The current treatment regimens for patients with T1DM or T2DM aim to treat insulin resistance, lower inflammation and reduce oxidative stress, which all contribute to the pathogenesis of DiaCM [13]. We hope that the development of an optimal animal model will aid in increasing the understanding of some pathophysiological mechanisms based on the accelerated progression of diabetic complications. Moreover, these models will continue to facilitate the discovery of novel targets and to advance unconventional treatment strategies for HF with DM patients, such as gene- or cell-based therapies [1,5,13,155].

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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