



Published in final edited form as:

Circ Res. 2018 February 16; 122(4): 624–638. doi:10.1161/CIRCRESAHA.117.311586.

Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity

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Abstract

Heart failure and related morbidity and mortality are increasing at an alarming rate, in large part, because of increases in aging, obesity and diabetes. The clinical outcomes associated with heart failure are considerably worse for patients with diabetes than for those without diabetes. In persons with diabetes, the presence of myocardial dysfunction in the absence of overt clinical coronary artery disease, valvular disease and other conventional cardiovascular risk factors such as hypertension and dyslipidemia has led to the descriptive terminology, “diabetic cardiomyopathy”. The prevalence of diabetic cardiomyopathy is increasing in parallel with the increase in diabetes. Diabetic cardiomyopathy is initially characterized by myocardial fibrosis, dysfunctional remodeling and associated diastolic dysfunction, later by systolic dysfunction, and eventually by clinical heart failure. Impaired cardiac insulin metabolic signaling, mitochondrial dysfunction, increases in oxidative stress, reduced nitric oxide bioavailability, elevations in advanced glycation end products and collagen – based cardiomyocyte and extracellular matrix stiffness, impaired mitochondrial and cardiomyocyte calcium handling, inflammation, renin angiotensin-aldosterone system activation, cardiac autonomic neuropathy, endoplasmic reticulum stress, microvascular dysfunction, and a myriad of cardiac metabolic abnormalities have all been implicated in the development and progression of diabetic cardiomyopathy. Molecular mechanisms linked to the underlying pathophysiological changes include abnormalities in AMP-activated protein kinase, peroxisome proliferator-activated receptors, *O*-linked N-acetylglucosamine, protein kinase C, microRNA, and exosome pathways. The aim of this review is to provide a contemporary view of these instigators of diabetic cardiomyopathy as well as mechanistically based strategies for the prevention and treatment of diabetic cardiomyopathy.

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Disclosures
None

Keywords

Diabetes; Cardiac fibrosis; Heart failure

Diabetic cardiomyopathy is defined by the existence of abnormal myocardial structure and performance in the absence of other cardiac risk factors such as coronary artery disease, hypertension, and significant valvular disease in individuals with diabetes. It was first described in 1972¹ in post-mortem pathological findings from four diabetic patients who manifested heart failure symptoms without evidence of coronary artery or valve disease and further confirmed in a 1974 Framingham Heart Study that demonstrated a higher incidence of heart failure in diabetic women (5 fold) and men (2.4-fold) after adjustment for other risk factors such as age, coronary heart disease, and hypertension.² In 2013, the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA)³ and the European Society of Cardiology (ESC) in collaboration with the European Association for the Study of Diabetes (EASD)⁴ defined diabetic cardiomyopathy as a clinical condition of ventricular dysfunction that occurs in the absence of coronary atherosclerosis and hypertension in patients with diabetes. In its early stages diabetic cardiomyopathy includes a hidden subclinical period characterized by structural and functional abnormalities, including left ventricular (LV) hypertrophy, fibrosis and cell signaling abnormalities. These pathophysiological changes of cardiac fibrosis and stiffness and associated subclinical diastolic dysfunction often evolve to heart failure with normal ejection fraction (HFpEF) and eventual systolic dysfunction accompanied by heart failure with reduced ejection fraction (HFrEF). This review summarizes recent research exploring molecular mechanisms, structural and functional changes and possible therapeutic approaches for the prevention and treatment of diabetic cardiomyopathy. It also highlights unmet needs and future research directions to better understand fundamental molecular abnormalities which promote this cardiomyopathy.

Clinical aspects of diabetic cardiomyopathy

Epidemiology of diabetes related heart failure

Clinical trials show the prevalence of heart failure in diabetic patients to range from 19% to 26%.⁵⁻⁷ The Framingham Heart Study found the incidence of heart failure was increased in both male and female diabetic patients when compared with age-matched individuals and this association was independent of obesity, hypertension, dyslipidemia, and coronary heart disease.² One study found that the incidence of heart failure was higher in diabetic (39%) compared with non-diabetic (23%) patients, with a relative risk of 1.3 for developing heart failure following 43 months of observation.⁸ Further data derived from a population-based observational study in the Cardiovascular Health Study (CHS), the Strong Heart Study (SHS),⁹ and the Multi-Ethnic Study of Atherosclerosis (MESA)¹⁰ demonstrated differences in LV mass and wall thickness, and increased diastolic and systolic dysfunction between diabetic patients and normal individuals. Meanwhile, in type 1 diabetes each 1% increase in glycated hemoglobin A_{1c} (HbA_{1c}) was linked to a 30% increase for risk of heart failure¹¹ whereas in type 2 diabetes each 1% rise in HbA_{1c} levels was associated with an 8% increase in risk, independent of other risk factors including obesity, smoking, hypertension,

dyslipidemia, and coronary heart disease,¹² suggesting that graded increases in glycemia are a powerful promoter of heart failure in diabetic patients.

Risk factors for diabetic cardiomyopathy

Hyperglycemia, systemic insulin resistance and impaired cardiac insulin metabolic signaling are major clinical abnormalities in diabetes, and all are involved in the pathogenesis of diabetic cardiomyopathy (Fig 1 and 2).¹³ In a prospective national survey of heart failure patients, 1811 individuals with and 2182 without pre-existing diabetes, glucose levels of 110–140, 140–200 and 200 mg/dL were associated with a 9%, 16% and 53% increased mortality risk when compared to an admission blood glucose < 110 mg/dL in patients with no pre-existing diabetes. There was a linear relationship between blood glucose level and long-term mortality in heart failure even in patients without a clinical diagnosis of diabetes. On the other hand, increased mortality risk was seen only in diabetics with glucose levels >200 mg/dL.¹⁴ In the UKPDS clinical study, a 1% reduction in HbA_{1c} was associated with a 16% risk reduction for development of heart failure,¹² suggesting that there is a time related log-linear relationship between long-term glycemic control and heart failure risk. Further, after 4 years of follow-up, a community based study of 6814 persons with no initial coronary artery disease demonstrated that increasing indices of metabolic syndrome tracked with increasing heart failure risk, with two-thirds of these patients developing HFpEF.¹⁵ The contemporary increase in dietary refined carbohydrate, and especially fructose, consumption may also impact development of diabetic cardiomyopathy as described in more in detail in the molecular mechanisms component of this review.¹⁶

Evolution of diabetic cardiomyopathy to clinical heart failure

Diabetic cardiomyopathy is usually asymptomatic in the early stages of its evolution.¹³ One of the earliest manifestations is LV hypertrophy and/or decreased LV compliance characterized by impaired early diastolic filling, increased atrial filling, and prolonged isovolumetric relaxation.¹³ LV dilation and symptomatic heart failure occur after the development of systolic dysfunction.¹³ Indeed, our recent data support the notion that diastolic dysfunction, as observed by cine magnetic resonance imaging in rodents, is associated with impaired cardiac insulin metabolic signaling.¹⁷ Cardiomyocyte stiffness and hypertrophy, as well as myocardial fibrosis all contribute to this cardiac abnormality (Fig. 1). The Cardiovascular Health Study found that, in a cohort of 5201 men and women, the ventricular septal and left posterior myocardial wall thicknesses were greater in diabetic patients than in nondiabetic individuals and that this was associated with compromised systolic or diastolic function.¹⁸

There is a considerable body of epidemiological evidence that implicates obesity, linked to increased intake of refined carbohydrates and decreased exercise, in the increasing prevalence of diabetes and related heart disease throughout the world. Lifestyle changes such as aerobic exercise, weight control and smoking cessation are efficacious therapeutic approaches in the prevention of diabetic cardiomyopathy. Sustained glycemic control reduces the prevalence of diabetic cardiomyopathy and reduces cardiovascular disease (CVD). For example, normalization of glycaemia with insulin therapy reduced cardiomyocyte hypertrophy, collagen content and diastolic dysfunction and limited

progression of diabetic cardiomyopathy in type 1 diabetic rodent models.¹⁹ There is emerging evidence that some glycemic therapies may have specific benefits. In a retrospective cohort study of 10,920 patients, metformin use was associated with a low risk of mortality in diabetic individuals with heart failure.²⁰ Sodium–glucose cotransporter (SGLT) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists have beneficial effects on CVD outcomes in type 2 diabetic patients.¹³ A meta-analysis of randomized controlled trials found that dipeptidyl peptidase 4 inhibitors and peroxisome proliferator-activated receptor (PPAR) agonists increased the risk of heart failure in patients with or at risk for type 2 diabetes mellitus. The EMPA-REG OUTCOME trial showed that SGLT2 antagonist treatment with empagliflozin reduced primary outcomes such as nonfatal myocardial infarction, nonfatal stroke, and CVD related mortality in 7020 diabetic patients.²¹ Finally, treatment with two long acting GLP-1 receptor agonists significantly reduced CVD events and heart failure in high risk diabetic patients.^{22, 23} The results suggest that these anti-diabetic drugs may have a role in preventing and treating diabetic cardiomyopathy and associated CVD in type 2 diabetic patients.

Functional phenotype of diabetic cardiomyopathy

The first stage of diabetic cardiomyopathy is clinically asymptomatic and is characterized by increased fibrosis and stiffness; there is a reduction of early diastolic filling and an increase in atrial filling and enlargement, as well as an elevated LV end-diastolic pressure.²⁴ Underlying pathological factors include hyperglycemia, systemic and cardiac insulin resistance, increased free fatty acid (FFA) levels, systemic and tissue inflammation, oxidative stress and activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS) (Fig 1).¹³ Reduced calcium (Ca^{2+}) pump activity-induced inefficient sequestration of sarcoplasmic reticulum Ca^{2+} is regarded as an important contributor to the development of the cardiac diastolic dysfunction.²⁵

The second stage of diabetic cardiomyopathy is characterized by LV hypertrophy, cardiac remodeling, advancing cardiac diastolic dysfunction, and the consequent emergence of clinical indications of HFpEF.¹³ With progression of diabetic cardiomyopathy, diastolic dysfunction and reduced cardiac compliance may co-exist with systolic dysfunction leading to reduced ejection fraction, prolonged pre-ejection performance, an enlarged LV chamber, shortened ejection period, and the latter by an increased resistance to filling with increased filling pressures.¹³ Abnormalities in contractile and regulatory protein expression are responsible for the mechanical defects in cardiac contraction. For example, decreased Ca^{2+} sensitivity along with shifts in cardiac myosin heavy chain from V1 to V3 isoforms contributes to the impaired cardiac systolic dysfunction, a long-term complication of diabetes.²⁶ Phosphorylation of troponin also contributes to depressed myocardial contractility since myosin light chain-2 and troponin I are involved in regulating cardiomyocyte contraction.²⁷

The phenotypes and underlying mechanisms of diabetic cardiomyopathy in type 2 diabetes have been mostly investigated in *db/db* mice, *ob/ob* mice, Zucker diabetic fatty rats, and diabetic patients.¹³ The impact of type 1 diabetes on systolic and diastolic function is less clear. As in type 2 diabetes, diastolic dysfunction is also often observed in type 1 diabetes.²⁸

The underlying mechanisms of diabetic cardiomyopathy in type 1 diabetes probably mostly overlap but different alterations exist in hearts of type 2 diabetes.²⁹ For instance, systolic function was preserved and cardiac hypertrophy was not observed in type 1 Akita diabetic mice, although hearts were smaller compared to non-diabetic controls.³⁰ Cardiomyocyte autophagy was enhanced in type 1 but suppressed in type 2 diabetes.²⁸ Clearly, further studies are necessary to understand the potential differences in phenotype and underlying mechanisms for diabetic cardiomyopathy in type 1 and type 2 diabetes.

Thus, cardiac dysfunction in diabetic hearts progresses from subclinical cardiac abnormalities such as LV fibrosis to diastolic dysfunction and eventually systolic dysfunction accompanied by reduced ejection fraction. A number of non-invasive techniques including echocardiography, computed tomography, and cinematic magnetic resonance imaging have been applied to detect changes of cardiac structure (i.e. fibrosis) and function.¹³ Further, elevated levels of atrial natriuretic peptide, brain natriuretic peptide, and *O*-linked N-acetylglucosamine (*O*-GlcNAc), among others, may also serve as markers for diabetic cardiomyopathy and heart failure.¹³

Molecular mechanisms underlying diabetic cardiomyopathy

Cardiac structural abnormalities

The mechanism promoting cardiomyocyte stiffness in the diabetic heart include impaired insulin metabolic signaling that decreases glucose transporter type 4 (GLUT4) recruitment to the plasma membrane and glucose uptake, thus lowering sarcoplasmic reticulum Ca^{2+} pump activity and increasing cardiomyocyte intracellular Ca^{2+} .¹³ Meanwhile, abnormal insulin metabolic signaling also decreases insulin-stimulated coronary endothelial nitric oxide (NO) synthase (eNOS) activity and NO production increasing cardiomyocyte intracellular $\text{Ca}^{2+}/\text{Ca}^{2+}$ sensitization and reducing sarcoplasmic Ca^{2+} uptake.¹³ Reduction of NO bioavailability may also lead to phosphorylation of titin increasing the ratio of stiff titin isoform N2B/N2BA (compliant) expression. These pathophysiological abnormalities increase cardiac stiffness and impair relaxation, cardinal manifestations of diabetic cardiomyopathy.¹³ Other pertinent abnormalities include hyperglycemia, insulin resistance and oxidative stress that promote expression of a number of cardiomyocyte hypertrophic genes such as β -myosin heavy chain, insulin-like growth factor 1 (IGF-1) receptor, and B-type natriuretic peptide.³¹ High insulin levels induce cardiomyocyte hypertrophy by binding to the IGF-1 receptor. IGF-1, produced by cardiomyocytes, can also stimulate cardiomyocyte hypertrophy via the insulin receptor, extracellular signal-regulated kinase 2 (Erk1/2) and phosphatidylinositol 3-kinase (PI3K) signaling pathways.³² Crosstalk between the IGF-1 and insulin signaling pathways plays an important role in hyperglycemia/insulin resistance-induced cardiac hypertrophy and fibrosis in diabetic cardiomyopathy, as described in more detail later in this review.

Development of myocardial fibrosis in diabetic cardiomyopathy involves the deposition of stiff collagen and its cross linking, cardiac interstitial fibrosis, progressive abolition of muscular fibrils, perivascular fibrosis, thickened and sclerotic small coronary vessels, and basement membrane thickening, as well as coronary microvascular sclerosis and microaneurysms.^{33, 34} Activation of the RAAS and SNS, stimulation of advanced glycation

end products (AGE)-mediated signaling via RAGE (cell surface receptor for AGE), hyperinsulinemia and hyperglycemia collectively lead to activation of transforming growth factor beta 1 (TGF- β 1) pathway and dysregulation of extracellular matrix (ECM) degradation.³⁵ Some biomarkers of collagen synthesis, including inflammation cytokines, connective tissue growth factor, metalloproteinases, and galectin-3 can be used clinically in the determination of myocardial fibrosis.³⁶ As discussed in more detail later, reduced bioavailable NO, increased oxidative stress, and an activated TGF- β 1/SMAD signaling pathway, in concert with impaired insulin metabolic signaling pathways, increase the myocardial fibronectin and collagen content and cardiac interstitial fibrosis characteristic of diabetic cardiomyopathy.¹⁷

Cardiac insulin resistance and diabetic cardiomyopathy

Cardiac insulin signaling mediates cellular homeostasis via control of protein synthesis, substrate utilization, and cell survival. Glucose transport in cardiac tissue is mediated via GLUT4 as in skeletal muscle, liver and fat tissue. Insulin, binding to the insulin receptor, activates insulin signaling/docking molecule insulin receptor substrate (IRS)-1/2 and downstream PI3K/protein kinase B (Akt), stimulating GLUT4 translocation to the cell membrane and subsequent glucose uptake.¹³ Further, normal coronary artery and myocardial insulin metabolic signaling promotes eNOS activation and bioavailable NO necessary for optimal coronary microvascular flow and myocardial function.^{13, 17} Cardiac insulin receptor knockout decreases cardiac glucose uptake, increases cardiac reactive oxygen species (ROS) production, and induces mitochondrial dysfunction.^{37, 38} Double IRS-1/2 knockout reduces cardiomyocyte adenosine triphosphate (ATP) content, impairs cardiac metabolism and function, and increases fibrosis and cardiac failure.^{37, 38} Reduced PI3K/Akt signaling and reduced GLUT4 expression and translocation have also been found in ventricular muscle biopsies obtained from patients with type 2 diabetes.³⁹ The E3 ubiquitin ligase, mitsugumin 53 (MG53), may play an important negative role in the maintenance of insulin signaling.⁴⁰ Elevated cardiac MG53 protein levels in a type 2 diabetic mouse model were correlated with increased proteosomal degradation of the insulin receptor and IRS-1. Further, cardiomyocyte-specific overexpression of MG53 inhibited insulin signaling and increased cardiac fibrosis,⁴¹ suggesting down-regulation of cardiac MG53 may be a potential therapeutic strategy in the prevention of diabetic cardiomyopathy and/or progression to clinically manifested heart failure.

Risk factors such as obesity and inappropriate activation of RAAS can impair cardiac insulin metabolic signaling through enhanced activation of the mammalian target of rapamycin (mTOR)/S6 kinase 1/(S6K1) signaling pathway,^{13, 17} which increases serine phosphorylation and reduces tyrosine phosphorylation of IRS-1/2 and impairs PI3K engagement and Akt/eNOS activation and NO production.^{13, 17} Lower NO production impairs coronary vessel relaxation and insulin-mediated capillary recruitment, both of which are important for the delivery of insulin and glucose necessary for normal myocardial energetics.^{42–45} Impairment of NO production also leads to increased activation of collagen cross linking enzymes such as transglutaminase thereby promoting cardiac fibrosis and stiffness.^{1,10} Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), have been reported to induce cardiac insulin resistance through activation of nuclear factor

kappa-light-chain-enhancer of activated B cells (NF- κ B) and c-Jun N terminal kinase (JNK) that induce phosphorylation of IRS-1.¹³ Activation of FoxO1 (forkhead box-containing protein, O subfamily) directly regulates IRS1 signaling and decreases PI3K/Akt signaling, leading to insulin resistance in mice fed a high fat diet.⁴⁶ Further, deletion of cardiac FoxO1 largely prevented heart failure in these animals.⁴⁷ Thus, FoxO1 may also provide a novel therapeutic or preventive strategy for treating individuals with diabetic cardiomyopathy.⁴⁷

Role of fructose in the pathogenesis of diabetic cardiomyopathy

High fructose diets induce cardiomyocyte autophagy, oxidative stress, and impaired insulin metabolic PI3K/Akt/eNOS signaling and interstitial fibrosis.^{16, 48} Generally, fructose is readily absorbed and rapidly metabolized by the human liver through GLUT2 and 5.¹³ Fructose 1-phosphate is cleaved to dihydroxyacetone phosphate by aldolase B, which can then be isomerized into glyceraldehyde 3-phosphate and acetyl-coenzyme A (CoA). Acetyl-CoA is either oxidized in the tricarboxylic acid cycle or directed towards FFA synthesis.^{49, 50} Phosphorylation of fructose also decreases ATP production.^{49, 50} Enhanced cellular fructose metabolism promotes a number of subcellular hexose sugar-related protein modifications, such as *O*-GlcNAc, and formation of advanced glycation end products (AGE) that contribute to impaired insulin metabolic signaling, reductions in NO production, and increased cardiac fibrosis.⁵¹

Decreased flexibility in substrate utilization in diabetic cardiomyopathy

Under normal physiological circumstances, the heart displays considerable metabolic substrate flexibility, utilizing energy from various substrates such as FFAs, glucose, ketone bodies, lactate, and some amino acids to produce ATP, the predominant source of energy for cardiac energetics.¹³ Mitochondria normally occupy approximately 20 to 30 % of the total cell volume of cardiomyocytes.¹³ Typically, mitochondrial oxidative phosphorylation produces more than 95% of ATP.⁵² The citric acid cycle usually accounts for the remaining 5% of ATP produced from glucose and lactate in the heart.⁵³ However, in the setting of hyperglycemia, insulin resistance and hypertriglyceridemia, there is a reduction in the myocardium's ability to use glucose as an energy source, and it subsequently switches to FFAs.⁵³ This energy substrate switch is accompanied by impaired oxidative phosphorylation and a mitochondrial proton leak that results in increased production of ROS. As the heart has very limited anti-oxidant capacity, increased mitochondrial ROS production leads to NO destruction and reduced bioavailable NO, hallmarks of diabetic cardiomyopathy.^{1, 22}

The role of abnormal FFA metabolism in diabetic cardiomyopathy

Increased FFA release from adipose tissue and increased capacity of myocyte sarcolemmal FFA transporters also contribute to the development of diabetic cardiomyopathy.^{13, 53} Cluster of Differentiation 36 (CD36), a predominantly membrane-located protein and transporter that promotes FFA uptake in both sarcolemma and endosomal membranes, is increased in diabetic hearts.⁵⁴ Increased subcellular vesicular recycling of CD36 from endosomes to the plasma membrane increases the rate of cellular FFA uptake in diabetic hearts.^{13, 54} CD36 is also paramount in AMP-activated protein kinase (AMPK)-mediated stimulation of FFA uptake in cardiomyocytes. Indeed, CD36-knockout mice show a 70% reduction in FFA uptake in cardiomyocytes⁵⁵ and CD36 deficiency rescues lipotoxic

cardiomyopathy.^{56, 57} Activation of AMPK is responsible for early activation of glucose uptake and glycolysis and improves cardiac function in diabetic patients.⁵⁸ However, AMPK activation is attenuated in diabetes increasing FFA uptake and triacylglycerol accumulation and reducing glucose utilization, also characteristic of diabetic cardiomyopathy.⁵⁹

Several lipid metabolites such as diacylglycerols (DAGs) and ceramides impair insulin metabolic signaling contributing to and exacerbating diabetic cardiomyopathy. Insulin sensitivity in obese humans can be correlated to elevated DAG content and protein kinase C (PKC) ϵ activation.⁶⁰ DAG increases lipid-associated endoplasmic reticulum stress.⁶⁰ A high fat diet increases DAG in the membrane fraction, activating PKC ϵ and inducing insulin resistance,⁶¹ and lowering NO production.⁶² Comparative gene identification 58 (CGI-58) is a lipid droplet-associated protein that promotes triglyceride hydrolysis and adipose triglyceride lipase activation.⁶³ Inhibition of CGI-58 induced hepatic steatosis and increased total DAG content in the absence of hepatic insulin resistance.⁶³ Studies have found that CGI-58 gene knock out prevents DAG accumulation at the plasma membrane, PKC ϵ activation, and the impairment of hepatic insulin metabolic signaling.^{61, 63} Meanwhile, ceramide can directly activate atypical PKCs and inhibit insulin metabolic Akt/PKB signaling, attenuating GLUT4 translocation and insulin-stimulated glucose uptake in diabetic hearts⁶². These data support a role for intracellular compartmentation of DAG and ceramide in causing lipotoxicity and metabolic insulin resistance in a number of tissues including the heart.

Abnormalities in ketogenesis in diabetic cardiomyopathy

Type 2 diabetes is often associated with decreased ketogenesis because of systemic insulin resistance and hyperinsulinemia.⁶⁴ Ketones, such as B-hydroxybutyrate, may play a key role in maintaining bio-energetic homeostasis in diabetic cardiomyopathy where there is reduced cardiac glucose utilization.⁶⁵ In this regard, treatment with empagliflozin, a SGLT2 antagonist, increased ketone levels providing a more efficient energy source in the failing myocardium of diabetic patients with heart failure,⁶⁶ perhaps compensating for an impairment in mitochondrial energy transduction related to decreased myocardial glucose utilization.

Abnormalities in cardiac glucose FFA cycling

The glucose fatty-acid cycle (Randle cycle) is a metabolic mechanism which involves competition between glucose and FFAs for their oxidation and uptake and is thus related to insulin resistance and type 2 diabetes. In this regard, activation of lipolysis provides FFAs as the preferred fuel source leading to ketogenesis during fasting whereas inhibition of glucose oxidation contributes to a glucose-sparing effect that is an essential survival mechanism during times of starvation.⁶⁷ In the fed state or during exercise, glucose is rerouted to glycogen and muscle glycogen content is increased.⁶⁷ Typically, FFA oxidation increases the mitochondrial ratios of acetyl-CoA/CoA and nicotinamide adenine dinucleotide (NADH)/NAD⁺ that inhibit pyruvate dehydrogenase activity and impair glucose metabolism, resulting in accumulation of cytosolic citrate, increased glucose 6-phosphate and inhibition of hexokinase.^{67, 68} Meanwhile, glucose oxidation produces citrate that can be metabolized

to malonyl-CoA; malonyl-CoA controls the entry and oxidation of long chain FFA favoring FFA esterification.^{67, 69, 70}

Mitochondrial dysfunction in the genesis of diabetic cardiomyopathy

Mitochondrial dysfunction plays a pivotal role in the development of diabetic cardiomyopathy and associated heart failure.⁷¹ Mitochondrial oxidative phosphorylation provides 90% of intracellular ATP production in cardiomyocytes but in type 2 diabetes, mitochondria switch from glucose to FFA oxidation for ATP production (Fig. 1).¹³ This is accompanied by increased mitochondrial ROS generation and impaired oxidative phosphorylation. Altered mitochondrial Ca^{2+} handling further promotes mitochondrial respiratory dysfunction leading to cell death.⁷² Metabolic stress-induced mitochondrial dysfunction also increases Ca^{2+} overload-induced opening of the mitochondrial permeability transition pores resulting in cardiomyocyte autophagy and cardiac necrosis.⁷³

Mitochondrial oxidative stress in the pathogenesis of diabetic cardiomyopathy

Oxidative stress promotes development and progression of cardiac insulin resistance, diabetic cardiomyopathy and heart failure (Fig. 1). Mitochondrial ROS are a natural byproduct of oxygen metabolism at complexes I and III within the electron transport chain.¹³ Under normal physiological conditions, the major electrochemical proton gradient is utilized to synthesize ATP.¹³ However, hyperglycemia and insulin resistance increase the NADH and flavin adenine dinucleotide flux to the mitochondrial respiratory chain, resulting in hyperpolarization of the mitochondrial inner membrane, inhibition of electron transport in complex III and excess ROS production.⁷⁴ Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase is another important source of cardiomyocyte ROS. Increased cardiomyocyte NADPH oxidase activity, has been reported in diet-induced obesity and systemic and cardiac insulin resistance.⁷⁵ Increased RAAS-mediated NADPH oxidase activity may also directly promote cardiac fibrosis by activation of a pro-fibrotic TGF- β 1/Smad 2/3 signaling pathway.^{76, 77} Other sources of ROS in diabetic cardiomyopathy include increases in xanthine oxidase, and microsomal P-450 enzyme activity, and uncoupling of NO synthase. Elevated cardiac tissue ROS not only increases polyol pathway flux, formation of AGEs, expression of the receptor for AGEs and its activating ligands, PKC signaling, and the hexosamine pathway, but also inhibits eNOS and prostacyclin synthase activity all of which contribute to the development of cardiomyopathy as previously reviewed.⁷⁸

Role of AGEs and RAGE in the pathophysiology of diabetic cardiomyopathy

Hyperglycemia increases AGE accumulation and induces myocardial structural alterations by increases in nonenzymatic glycation, oxidation of lipids and proteins, myocardial collagen and fibronectin production and connective tissue cross-linking and fibrosis (Fig. 1).¹³ Indeed, AGE-induced connective tissue cross-linking of ECM promotes myocardial fibrosis and impaired passive relaxation.¹³ AGEs may also bind to RAGE which further promotes maladaptive structural changes and impaired myocardial energetics.¹³ For example, interaction of AGEs with RAGE on cardiomyocyte surfaces induces maladaptive pro-inflammatory responses and increases matrix protein and connective tissue production, mediated through Janus kinase (JAK) and mitogen-activated protein kinase (MAPK) pathway activation.¹³ In addition, AGEs elevation increases ROS production and TGF- β 1/

SMAD pathway activation, and connective tissue production and fibrosis. In a prospective clinical study of 194 patients with acute coronary syndrome, AGEs were an independent risk factor in post-infarction heart failure.⁷⁹ Further, increases in plasma AGEs independently predicted mortality and hospitalization for heart failure in a study of 580 diabetic patients.⁸⁰

Impaired mitochondrial Ca²⁺ handling in diabetic cardiomyopathy

Cytosolic Ca²⁺ levels regulate cellular metabolism, muscle contraction, and cell signaling (Fig. 1). Typically, in cardiac excitation–contraction coupling, Ca²⁺ enters the cytoplasm through voltage sensitive L-type Ca²⁺ channels after depolarization of the sarcolemma and this triggers Ca²⁺-release from the sarcoplasmic reticulum. The Ca²⁺ binds troponin C to induce myofibrillar contraction.^{81, 82} During cardiac relaxation, Ca²⁺ is transported back into the sarcoplasmic reticulum and the remaining cardiomyocyte Ca²⁺ is pumped out by the sarcolemma Na⁺/Ca²⁺ exchanger and the plasma membrane Ca²⁺ pump.^{81, 82} However, in diabetic cardiomyopathy impaired Ca²⁺ handling by all of these transporters increases action potential duration and prolongs diastolic relaxation time.¹³ Elevated intracellular resting Ca²⁺, prolongation of intracellular Ca²⁺ decay, slowed Ca²⁺ transients, reduction of sarcoplasmic reticulum Ca²⁺ pumping, and impairment of sarcoplasmic reticulum Ca²⁺ reuptake have been documented in hearts of type 2 diabetic mice.^{83, 84} Similar changes are seen in type 1 diabetic rodent models.^{85, 86} The data suggest that impaired cardiomyocyte Ca²⁺ handling plays a key role in the development of the cardiac diastolic dysfunction characteristic of early diabetic cardiomyopathy.

Inflammation as an instigator of diabetic cardiomyopathy

A maladaptive pro-inflammatory response has been implicated in the development of diabetic cardiomyopathy. The innate immune system, i.e. neutrophils, mast cells, dendritic cells, macrophages, and eosinophils, is involved (Fig 1).¹³ Activation and expression of pro-inflammatory cytokines such as TNF α , interleukins (IL) 6 and 8, monocyte chemoattractant protein 1 (MCP-1), adhesion molecule intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 all contribute to cardiac oxidative stress, remodeling and fibrosis, and diastolic dysfunction.¹³ Cytokine expression is regulated by the nuclear transcription factor, NF- κ B.¹³ Finally, the Toll-like receptor-4 also plays an important role in triggering increased NF- κ B, pro-inflammatory and innate immune system responses.⁸⁷ These pro-inflammatory responses occur in different populations of cardiac cells including coronary endothelial and smooth muscle cells as well as fibroblasts and cardiomyocytes.

High FFA levels, impaired insulin metabolic signaling, and hyperglycemia activate the NLRP3 inflammasome, a novel molecular marker in diabetic cardiomyopathy.⁸⁸ Separation from cytoplasmic chaperones and oligomerization follows NLRP3 activation, leading to recruitment of procaspase-1.⁸⁸ Activated caspase-1 processes IL-1 β and IL-18 precursors and serves as an enhancer of multiple pro-inflammatory pathways involving NF- κ B, chemokines, and ROS. A NF- κ B positive feedback loop further increases NLRP3 inflammasome assembly and pro-caspase-1 activation, and pro-IL-1 β processing and maturation.⁸⁸ Meanwhile, increased monocyte/macrophage migration through the coronary endothelium increases resident cardiac macrophages, which can be polarized into pro-inflammatory M1 phenotypes under conditions of increased ROS and reduced bioavailable

NO.^{13, 17} In recent investigative work it has been observed that macrophage pro-inflammatory M1 polarization is upregulated whereas macrophage M2 anti-inflammatory response is repressed in diabetic heart tissues.^{13, 17}

Activated RAAS in the genesis of diabetic cardiomyopathy

Increased activation of the systemic and tissue RAAS in states of hyperglycemia and insulin resistance plays an important role in the pathogenesis of diabetic cardiomyopathy and heart failure (Fig 1). Serum angiotensin II (Ang II) levels are significantly correlated with postprandial glucose concentrations in insulin resistance and type 2 diabetes.⁸⁹ Further, the pro-inflammatory Ang II receptor 1 (AT-1R) is upregulated and the anti-inflammatory AT-2R is downregulated in early diabetes.⁹⁰ Exercise induces a shift of the RAAS toward the angiotensin converting enzyme 2/Mas receptor axis in skeletal muscle, providing protection in obese rats.⁹¹ Conversely, inhibition of the AT2 receptor with PD123319 impairs insulin signaling in C57BL/6 mice.⁹² In patients with type 1 diabetes, hyperglycemia-induced activation of systemic RAAS appears to play a role in the pathogenesis of diabetic cardiomyopathy.^{93,94} Experimental evidence also supports a role for increased mineralocorticoids in systemic and tissue insulin resistance.¹⁷ High plasma aldosterone and overexpression of the tissue mineralocorticoid receptors (MR) are associated with systemic insulin resistance, hyperglycemia and dyslipidemia.⁹⁵ Large randomized controlled trials have shown that inhibition of the aldosterone/MR signaling pathway reduces morbidity and mortality in diabetic patients with both mild and moderately severe heart failure.⁶² RAAS activation impairs insulin metabolic signaling and induces systemic and cardiac insulin resistance, in part, through activation of the mTOR/S6K1 signaling pathway.⁹⁶ Meanwhile, enhanced AT-1R and MR activation increases coronary artery endothelial leukocyte/monocyte adhesion, pro-inflammatory cytokine expression, macrophage infiltration and polarization resulting in an increase in the pro-inflammatory M1 phenotype in the myocardium. These abnormalities exacerbate the maladaptive cardiac remodeling, interstitial fibrosis and diastolic dysfunction seen in diabetic cardiomyopathy.¹⁷

Autonomic neuropathy in diabetic cardiomyopathy

Cardiac autonomic neuropathy is a secondary complication related to sustained hyperglycemia (Fig. 1) and includes abnormalities in heart rate control, vascular hemodynamics, and cardiac structure and function.^{13, 97, 98} An early characteristic of cardiac autonomic neuropathy is reduction of parasympathetic activity with an imbalance toward relatively higher SNS activity.^{99, 10001} In this regard, activation of the SNS enhances beta-1 adrenergic receptor (β_1) signaling which promotes cardiac hypertrophy, interstitial fibrosis, cardiomyocyte apoptosis and impaired function.⁹⁸

Endoplasmic reticulum stress and increased cell death in diabetic cardiomyopathy

Cardiac oxidative stress, lipotoxicity, inflammation, and the accumulation of misfolded proteins impairs the function of cardiac endoplasmic reticulum and promotes endoplasmic reticulum stress, inducing the unfolded protein response (Fig. 1).¹³ Together, endoplasmic reticulum stress and the unfolded protein response inhibit cellular protein synthesis and degradation of misfolded or damaged proteins and ultimately increase cell apoptosis and autophagy.¹³ Increased cardiac apoptosis is a major risk factor for the development of

diabetic cardiomyopathy; biopsied diabetic heart tissue expresses 85-fold more cardiomyocyte apoptosis than control non-diabetic hearts.¹⁰¹ Endoplasmic reticulum stress also induces autophagy through a Ca^{2+} -dependent pathway, involving the inositol-requiring enzyme 1 (IRE1) and protein kinase RNA-like endoplasmic reticulum kinase (PERK) pathways.¹⁰² Typically, autophagy is regulated through the mTOR, AMPK, and silent information regulator (Sirt) pathways.¹³ mTORC1 has been proposed to regulate autophagy by repressing the autophagy related 1 (Atg1)–Atg13–Atg101/FIP200 (FAK family-interacting protein of 200 kDa) complex. Thus inhibition of mTORC1 facilitates the initiation of autophagy.¹⁰² The inositol requiring enzyme 1 arm of ER stress leads to JNK activation and increased phosphorylation of Bcl-2, which promotes its dissociation from Beclin-1.¹⁰² Thus, enhanced mTOR may serve as a convergence point for abnormalities involving the interplay between endoplasmic reticulum stress and autophagy in the hearts of diabetic individuals. However, in diabetic cardiomyopathy, dysregulation of autophagy impairs cardiomyocyte auto-phagosome and lysosome fusion.¹⁰³ One study found that chronic metformin treatment activated AMPK activity, restored autophagic activity, and inhibited cardiomyocyte apoptosis by disrupting the Bcl-2 and Beclin1 complex in diabetic heart tissue.¹⁰³ Therefore, the activation of the autophagic response is usually regarded as a compensatory feedback mechanism to protect against cell apoptosis and to maintain normal cellular function in conditions such as insulin resistance and type 2 diabetes.

Microvascular dysfunction in diabetic cardiomyopathy

Diabetic cardiomyopathy is classically defined in the context of absence of overt coronary artery disease. Nevertheless, diabetic cardiomyopathy may be associated with coronary microvascular dysfunction which impairs coronary blood flow and myocardial perfusion, ventricular function, and clinical outcomes including CVD.¹⁰⁴ In a clinical study of 2783 consecutive patients, impaired coronary flow reserve was associated with an adjusted 3.2- and 4.9-fold increase in the rate of cardiac death for both diabetic and nondiabetic individuals, respectively, indicating that coronary microcirculation dysfunction is a powerful, independent correlate of cardiac mortality among both diabetics and nondiabetics.¹⁰⁵ Treatment with an MR antagonist improved coronary microvascular function and prevented CVD in patients with type 2 diabetes suggesting an important role of cardiac MR activation in coronary microvascular dysfunction in diabetes.¹⁰⁶ Structural abnormalities in the coronary microcirculation include luminal obstruction, inflammation infiltration, vascular remodeling, and perivascular fibrosis.¹⁰⁷ Functional abnormalities in the coronary microcirculation include endothelial and smooth muscle cell dysfunction and impairment of vascular relaxation and constriction and ischemic reperfusion.¹⁰⁷ The normal function of coronary arteries, and downstream microcirculatory vessels, is impaired in diabetic cardiomyopathy (Fig. 1). Physiologically, a number of vasoactive substances, including NO, prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factors (EDHF), released from endothelial cells lining the coronary microcirculation, play a beneficial vasodilatory role.⁴² Indeed, although NO-mediated vasodilation may be impaired, vascular function in the early stages of diabetes is often preserved through normal or even enhanced EDHF-induced vasodilation.⁴² However, both NO- and EDHF-induced vasodilation are eventually affected leading to significant dysfunction of the microcirculation in the later stages of diabetes.⁴² Recent investigation has also suggested that persistently high plasma endothelin-1 (ET-1)

levels, along with reduced eNOS activity and NO production, are associated with the development of cardiac fibrosis and diastolic dysfunction in diabetic patients.¹⁰⁸ In this regard, endothelial cell-specific ET-1 knockout prevented diabetes-induced cardiac fibrosis and exerted a beneficial effect in the prevention of diabetic cardiomyopathy.¹⁰⁸

Molecular protein signature in diabetic cardiomyopathy

A number of molecular proteins and signaling pathways have been implicated as important contributors to the development of diabetic cardiomyopathy and heart failure. These include AMPK, PPARs, *O*-GlcNAc, SGLT2, PKC, MAPK, NF κ B, nuclear factor erythroid 2-related factor 2 (Nrf2), cyclic adenosine 5'-monophosphate-responsive element modulator (CREM), microRNAs (miRNA), and exosomes as discussed below (Fig 2).

Impaired AMPK activation in diabetic cardiomyopathy

AMPK is a master regulator of cellular energy homeostasis.¹³ With cellular stress and/or an increased AMP/ATP ratio, AMPK activation enhances expression and translocation of GLUT4 and thus insulin-induced glucose uptake, and increases mitochondrial biogenesis leading to FFA oxidation and glycolysis.⁵⁸ Specifically, in cardiomyocytes, activated AMPK positively stimulates glucose uptake, FFA oxidation, and glycolysis while negatively regulating mTOR signaling, gluconeogenesis, and lipid and protein synthesis.¹⁰⁹ Therefore, AMPK activation plays a beneficial role in preventing the progression of diabetic cardiomyopathy. For this reason, AMPK has been considered as a promising target for drug discovery and development for the prevention and reversal of diabetic cardiomyopathy.

Alterations in activation of cardiac PPARs

Several different PPAR isoforms, namely α , β/δ and γ , are expressed in the heart and play a key role in myocardial glucose and lipid metabolism and energy homeostasis. Importantly, they also exert roles not directly related to metabolism such as inflammation and oxidative stress.¹³ PPAR- α is expressed at relatively high levels in the heart and its activation directly impacts FFA uptake and mitochondrial FFA oxidation.¹³ PPAR- α regulates lipoprotein assembly and transport and modulates both oxidant and antioxidant defenses.¹¹⁰ Overexpression of cardiomyocyte-specific PPAR α causes decreased uptake of Ca²⁺ by the sarcoplasmic reticulum, LV hypertrophy, systolic dysfunction, and increased atrial natriuretic and B-type natriuretic peptide expression.⁵⁹ Conversely, deletion of cardiac PPAR- α prevents fasting-induced expression of FFA metabolic genes and induces a switch from FFA to glucose utilization.¹¹¹ With the development of diabetic cardiomyopathy, chronic exposure to elevated FFAs seems to reduce PPAR- α expression. In rodent cardiomyocytes, this was shown to further decrease cardiac function by inhibition of FFA oxidation and increased intracellular fat accumulation.^{112, 113} However, human studies suggest that PPAR- α expression is not significantly altered in the hearts of type 2 diabetes patients.^{72, 114} Activated PPAR- α -induced increases in FFA oxidation and utilization in the diabetic heart may thus initially serve as a compensatory mechanism to adjust substrate oxidation to available substrate supply. Further, reduced PPAR- α in advanced disease may have maladaptive consequences in terms of cardiac metabolism, including glucotoxicity and functional cardiac abnormalities. The role of PPAR- α in the development of cardiac

dysfunction in diabetic cardiomyopathy has been inadequately evaluated and needs to be further investigated. Similar to PPAR- α , PPAR- β/δ isoforms are also expressed abundantly in heart tissue and regulate transcriptional gene expression and FFA metabolism.¹¹⁵ Enhanced PPAR- β/δ signaling promotes FFA utilization whereas deletion of PPAR- β/δ decreases FFA oxidative gene expression and FFA oxidation.¹¹⁵ In addition, PPAR- γ plays important roles in cardiac anti-hypertrophic and anti-inflammatory effects.¹³ PPAR- γ agonists enhance cardiomyocyte insulin sensitivity and improve cardiomyocyte glucose uptake.¹³ Thus, PPAR- γ may be beneficial in maintaining glucose and FFA metabolism and cardiac function. Conversely, a deficiency in PPAR- γ signaling may contribute to the development of diabetic cardiomyopathy.

Increased O-GlcNAc in promoting cardiac fibrosis in diabetic cardiomyopathy

Hyperglycemia is associated with increased O-GlcNAcylation, which causes posttranslational modification of cardiac proteins (Fig 2). Sustained O-GlcNAc signaling exists in the diabetic heart and can exert detrimental effects that include decreases in mitochondrial function and energy generation, and increases in cardiac dysfunction and heart failure.¹¹⁶ Under physiological conditions, the hexosamine biosynthesis pathway drives a component of fructose-6-phosphate metabolism from glycolysis to generate the O-GlcNAc moiety.¹¹⁷ Transient activation of O-GlcNAc signaling is normally cytoprotective and increases cell survival.¹¹⁸ In contrast to transient upregulation, sustained elevation of O-GlcNAc signaling in the diabetic heart mediates diabetes-induced impairment of insulin metabolic signaling, cardiomyocyte apoptosis, myocardial excitation-contraction coupling, and cardiac relaxation.¹¹⁹ Overexpression of O-GlcNAcase removes O-GlcNAc and restores normal cardiomyocyte Ca²⁺ handling and cardiac function,¹²⁰ suggesting that targeting of hexosamine biosynthesis and O-GlcNAc may be a fruitful potential strategy for the prevention and therapy of diabetic cardiomyopathy.

SGLT2 abnormalities and potential cardiac benefits of inhibition of this transporter

Glucose is actively transported from the gut lumen into the gastrointestinal epithelium primarily by SGLT1, highly expressed in the brush-border membrane of enterocytes.^{121, 122} In hyperglycemia and insulin resistance, glucose and fructose absorption through the intestinal mucosa increases due to higher expression of SGLT1, GLUT2 and GLUT5 and increased brush border disaccharidases, sucrase, maltase and lactase activity.^{121, 122} SGLT2 is exclusively expressed in the kidney and mostly localized in the brush border membrane of proximal tubule epithelial cells in the S1 segment of the proximal convoluted tubule.^{121, 122} SGLT2 expression is significantly increased in diabetic humans¹²³, rats¹²⁴, and db/db mice¹²⁵ and this is correlated with glomerular hyperfiltration, increased glucose reabsorption and elevated plasma glucose.¹²⁶ Conversely, SGLT2 inhibition leads to natriuresis, osmotic diuresis, plasma volume contraction, and reduction of blood pressure and arterial stiffness, all mechanisms that may mitigate diabetic cardiomyopathy and heart failure. Further, SGL2 inhibitor treatment can shift cell metabolism from glucose to FA oxidation. Thus, the cardiac beneficial effects of SGLT2 may include making more of the ketone body, B-hydroxybutyrate, a highly energy efficient substrate for cardiac metabolism. Targeting SGLT2 has been shown to improve cardiovascular outcomes and mortality in type 2 diabetic

patients (Fig 2).²¹ Ongoing research is addressing the role of these agents for specific prevention and treatment of heart failure in diabetic individuals.¹²⁷

PKC activation promotes development of diabetic cardiomyopathy

PKC signaling pathways are activated in diabetic cardiomyopathy in response to hyperglycemia and insulin resistance (Fig 2). Oxidative stress, inflammation, and enhanced RAAS and SNS activity further promote PKC activation. To date, approximately 15 isoforms of PKC have been described in humans. These isoforms can be divided into 3 subfamilies based on second messenger signaling and particular mode of activation.^{128, 129} PKC α , β , ϵ , θ , and δ isoforms have been proposed to be involved in the development of diabetic cardiac hypertrophy.^{128, 129} For example, PKC β 2 has been shown to mediate hyperglycemia-induced diastolic cardiac dysfunction in diabetic rats through alterations in caveolin-3 expression and insulin metabolic Akt/eNOS signaling.¹²⁸ Further supporting its relevance, targeted inhibition of PKC β 2 in a transgenic mouse model of diabetic cardiomyopathy has been reported to improve fractional shortening and reverse cardiac hypertrophy and fibrosis.¹³⁰ Collectively, the data suggest that activation of PKC can induce cellular and functional changes that contribute to the development of diabetic cardiomyopathy and heart failure.

Role of MAPK and JNK activation in the genesis of diabetic cardiomyopathy

MAPK activation has also been implicated in the pathogenesis of diabetic cardiomyopathy and heart failure. Erk1/2, p38 MAPK and JNKs are three important MAPK subfamilies that regulate cardiac growth, hypertrophy, and remodeling.¹³ Enhanced myocardial phosphorylation of Erk 1/2 and activation of p38 MAPK occurs during ischemia in streptozotocin-induced diabetic models.¹³¹ Our research and that of others has demonstrated that obesity/insulin resistance-induced cardiac dysfunction is associated with enhanced S6K1 and Erk1/2 signaling.^{17,132} JNK can be activated by oxidative stress, inflammatory cytokines, and sphingolipid metabolites.¹³³ In turn, enhanced JNK signaling in the diabetic heart contributes to oxidative stress, endoplasmic reticulum stress, and interstitial fibrosis.¹³³ In contrast, inhibition of JNK phosphorylation by a curcumin analog prevents high glucose-induced inflammation and apoptosis in diabetic hearts.¹³³ In addition to these observations, JNK may play an important role in cardiomyocyte apoptosis.¹³⁴ As such, JNK activation was shown to cause increased cardiomyocyte apoptosis as early as days 3 and 7 in a type 1 diabetic rodent model.¹³⁴ Collectively, activation of both MAPK and JNK signaling appears to contribute significantly to the development of diabetic cardiomyopathy.

Role of NF- κ B activation in the genesis of diabetic cardiomyopathy

NF- κ B is one of the key transcription factors that regulate the expression of pro-inflammatory cytokines, pro-fibrotic genes, and cell survival¹³⁵ thus contributing to mitochondrial and cardiac dysfunction in diabetic hearts. NF- κ B is found in the cytoplasm of non-stimulated cells. Upon stimulation, I κ B is phosphorylated and its p50/p65 subunits translocate to the nucleus and bind κ B nuclear elements.¹³⁵ In diabetes, ROS, AGEs, and an activated cardiac tissue RAAS can directly induce NF- κ B activation. This, in turn, promotes maladaptive immune responses and the release of pro-inflammatory cytokines, such as TNF- α , MCP-1, IL-6 and IL8.¹³ Activated NF- κ B in diabetic mouse hearts has been shown to be

associated with increased NADPH oxidase mediated generation of ROS, peroxynitrite and superoxide.¹³⁶ These processes lead to a reduction in bioavailable NO. Inhibition of NF- κ B with pyrrolidine dithiocarbamate improves mitochondrial structural integrity and inhibits oxidative stress, increasing ATP synthesis and NO bioavailability thereby restoring cardiac function in type 2 diabetes.¹³⁶

Abnormalities of Nrf2 related antioxidant actions in diabetic cardiomyopathy

Nrf2 is a leucine zipper protein that promotes the expression of antioxidant proteins such as hemoxygenase in response to oxidative stress (Fig 2). Nrf2 is predominantly regulated by its binding to the inhibitor, Keap1, which targets Nrf2 for ubiquitination and degradation thus maintaining low cellular levels of Nrf2.¹³⁷ Under oxidative stress, Nrf2 and its regulators undergo a variety of modifications that cause dissociation of Nrf2 from Keap1. Free Nrf2 can then bind to small Maf proteins in the nucleus to activate transcription.¹³⁷ Hyperglycemia and insulin resistance repress Nrf2 expression and activity through an Erk 1/2 mediated pathway that contributes to oxidative stress and insulin resistance in cardiomyocytes.¹³⁸ Restoration of Nrf2 activity prevents diabetes-induced lipid accumulation, inflammation, fibrosis, and associated cardiac dysfunction,¹³⁹ providing another potential strategy for the prevention of diabetic cardiomyopathy.

Role of the transcription factor CREM in the genesis of diabetic cardiomyopathy

CREM is a transcription factor that regulates cAMP signaling and cardiac gene expression (Fig 2). Members of the CREM family may promote fibrosis of the heart, especially in response to hyperglycemia and elevated FFAs.¹⁴⁰ For example, CREM expression is increased in cardiomyocytes of chronically hyperglycemic type 1 diabetic mice and this is accompanied by increased cardiac fibrosis.¹⁴¹ These adverse effects were associated with alterations in histone acetylation and alterations in miRNAs profiles suggesting that CREM activation may promote epigenetic as well as genetic modifications in cardiac proteins and mediate “glycemic memory” in on-going progression of diabetic cardiomyopathy.

Role of miRNAs in promotion of diabetic cardiomyopathy

Diabetic cardiomyopathy is associated with increased expression of miRNAs – a group of short single-stranded non-coding RNA molecules with an average length of 22 nucleotides. Importantly, miRNAs control the expression of transcriptional and post-transcriptional target genes through binding to the 3'-untranslated region and regulate mitochondrial function, ROS production, Ca²⁺ handling, apoptosis, autophagy, and fibrosis, all of which are regarded as important mechanisms in diabetes-induced cardiac hypertrophy, remodeling and fibrosis, as well as heart failure progression.^{142, 143} miR-15a, -21, -24, -29, -30d, -103, -126, -146a, -150, -191, -223, -320, -375, and -486 have all been reported to be increased in type 2 diabetic individuals.^{142, 143} In cardiac tissue of a type 1 diabetic rodent model, expression of miR-21, -24, -142-3p, -195, -199a-3p, -700, -705, -208, -221 and 499-3p was upregulated whereas expression of miR-1, -20a, -29a, -143, -220b, and -373 was downregulated.¹⁴⁴ miR103, 107, -143 and -181 play a role in insulin sensitivity and systemic glucose metabolism.^{145, 146} Increased expression of miR-454, 500, -142-3p/5p and 1246 has been identified in cardiac diastolic dysfunction.¹⁴⁷ Other miRNAs, such as miR-113a, -133a, -150

have been found to be involved in the regulation of cardiomyocyte hypertrophy and interstitial fibrosis.¹⁴⁸

Abnormalities of Exosomes in diabetic cardiomyopathy

There is a close relationship between nutrient metabolism and exosome release from cells such as cardiomyocytes. Exosomes are extracellular vesicles with a diameter ranging from 30 to 90 nm and are regarded as important mediators of cell- to-cell communication.²⁴ They contain a variety of biological components including lipids, miRNAs, proteins and transcription factors that regulate both normal physiological and pathophysiological effects.²⁴ Exosomes that function with glucose transporters and glycolytic enzymes to increase glucose uptake, glycolysis, and pyruvate production in ECs were released from cardiomyocytes after glucose deprivation.¹⁴⁹ In type 2 diabetic hearts, exosomes containing high levels of miR-320 are released from cardiomyocytes and transported to coronary endothelial cells, resulting in reduced NO production and inhibition of angiogenesis via decreases heat shock protein 20 (Hsp20).¹⁵⁰ However, HSP20-engineered exosomes have a beneficial role in the regulation of cardiomyocyte exosome secretion and restoration of hyperglycemia-induced cardiac dysfunction.¹⁵¹ Therefore, exosomes may not only act as biomarkers, but targeting exosomes may also be a potential therapeutic strategy in the prevention and or progression of diabetic cardiomyopathy.

Conclusion and future perspectives

The cardinal features of diabetic cardiomyopathy include cardiac stiffness, myocardial fibrosis and hypertrophy with cardiac diastolic dysfunction and subsequent progression to both systolic dysfunction and clinical heart failure. Importantly, hyperglycemia and systemic and cardiac insulin resistance are independently associated with the development and progression of cardiac dysfunction and heart failure in diabetes. From a mechanistic point of view, mitochondrial dysfunction, oxidative stress, increased formation and deposition of AGEs, impaired mitochondrial Ca²⁺ handling and function, inflammation, activation of RAAS and SNS, cardiac autonomic neuropathy, endoplasmic reticulum stress, microvascular dysfunction, and cardiac metabolic disorders are involved in the pathophysiological process. Underlying these pathophysiological events, evidence supports a role for a number of proteins and signaling pathways including AMPK, PPARs, O-GlcNAc, SGLT2, PKC, MAPK, NFκB, Nrf2, miRNA and exosomes. Although diabetic cardiomyopathy appears to have an extensive preclinical course and the pathophysiological changes appear to be induced by the metabolic alterations in diabetes mellitus, a formal definition for diabetic cardiomyopathy as a distinct clinical entity remains vague due to a lack of accepted diagnostic criteria and information on subclinical CVD in the early stages of diabetes. Currently, there is not a specific histological property, biochemical marker, or clinical manifestation for the definitive diagnosis of diabetic cardiomyopathy. Also, there are no prospective clinical trials to support that hyperglycemia or hyperinsulinemia independently increase the risk for development of diabetic cardiomyopathy in the absence of other risk factors such as obesity, coronary heart disease, and hypertension. Recent scientific evidence for the potential use of exosome and circulating miRNAs as biomarkers for detection of diabetic cardiomyopathy highlights emerging methods for the diagnosis and

prevention of diabetes and cardiomyopathy. The American Diabetes Association recommends screening patients with type 2 diabetes for the prevention of diabetic cardiomyopathy but the results of the recently published Detection of Ischemia in Asymptomatic Diabetics (DIAD) trial indicate that screening of asymptomatic patients with nuclear imaging does not improve cardiac event rates.¹⁵² Unfortunately, screening approaches including B-type natriuretic peptide, exercise stress testing, and echocardiographic assessment do not appear to be sufficiently sensitive to identify subclinical dysfunction in diabetic patients.¹⁵³ Therefore, further studies are vital to the understanding of the precise mechanisms involved in the initiation and progression of diabetic cardiomyopathy, and to the development of novel strategies to reduce the risk of heart failure in diabetic patients.

Acknowledgments

Sources of Funding

JRS received funding from NIH (R01 HL73101-01A and R01 HL107910-01) and the Veterans Affairs Merit System (0018). Dr. Jia received funding from American Diabetes Association (Innovative Basic Science Award #1-17-IBS-201). Dr. Hill received funding from NIH (R01HL085119).

Nonstandard Abbreviations and Acronyms

ACCF	American College of Cardiology Foundation
AGE	Advanced glycation end products
AHA	American Heart Association
Akt	Protein kinase B
AMPK	AMP-activated protein kinase
Ang II	Angiotensin II
AT-1R	Ang II receptor 1
ATP	Adenosine triphosphate
Ca²⁺	Calcium
CD 36	Cluster of Differentiation 36
CGI-58	Comparative gene identification 58
CHS	Cardiovascular Health Study
CoA	Coenzyme A
CREM	Cyclic adenosine 5'-monophosphate-responsive element modulator
CVD	Cardiovascular disease
DAGs	Diacylglycerols

DIAD	Detection of Ischemia in Asymptomatic Diabetics
EASD	European Association for the Study of Diabetes
ECM	Extracellular matrix
EDHF	Endothelium-derived hyperpolarizing factors
eNOS	Endothelial NO synthase
Erk1/2	Extracellular signal-regulated kinase 1/2
ESC	European Society of Cardiology
ET-1	Endothelin-1
FFA	Free fatty acid
FoxO1	Forkhead box-containing protein, O subfamily
GLP-1	Glucagon-like peptide 1
GLUT4	Glucose transporter type 4
HbA_{1c}	Hemoglobin A _{1c}
HFpEF	Heart failure with normal ejection fraction
HFrEF	Heart failure with reduced ejection fraction
Hsp20	Heat shock protein 20
IGF-1	Insulin-like growth factor 1
IL	Interleukins
IRE1	Inositol-requiring enzyme 1
IRS	Insulin receptor substrate
JAK	Janus kinase
JNK	c-Jun N terminal kinase
LV	Left ventricular
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein 1
MESA	Multi-Ethnic Study of Atherosclerosis
MG53	Mitsugumin 53
miRNA	MicroRNAs
MR	Mineralocorticoid receptors

mTOR	Rapamycin
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide-adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor 2
O-GlcNAc	O-linked N-acetylglucosamine
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
PGI₂	Prostacyclin
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
RAAS	Renin-angiotensin-aldosterone system
RAGE	Cell surface receptor for AGE
ROS	Reactive oxygen species
S6K1	S6 kinase 1
SHS	Strong Heart Study
SGLT	Sodium–glucose cotransporter
SNS	Sympathetic nervous system
TGF-β1	Transforming growth factor beta 1
TNF-α	Tumor necrosis factor-alpha

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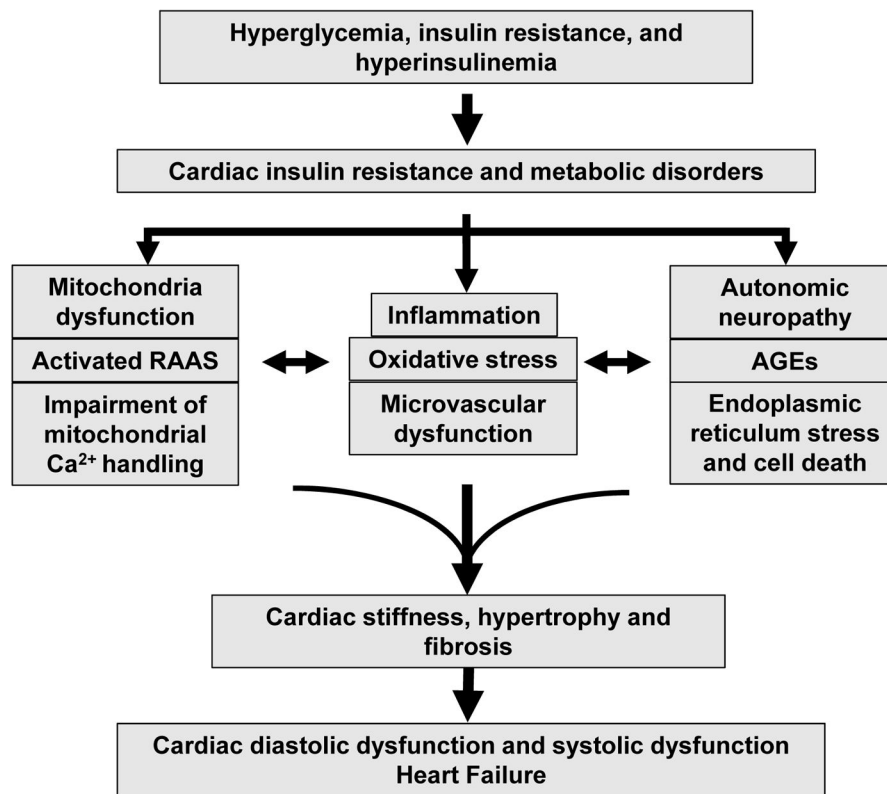


Fig. 1. Pathophysiological mechanisms of diabetic cardiomyopathy. Hyperglycemia, insulin resistance, and hyperinsulinemia induce cardiac insulin resistance and metabolic disorders that increase mitochondria dysfunction, oxidative stress, AGEs, impairment of mitochondria Ca^{2+} handling, inflammation, activation of RAAS, autonomic neuropathy, endoplasmic reticulum stress, cardiomyocyte death, as well as microvascular dysfunction. These pathophysiological abnormalities promote cardiac stiffness, hypertrophy, and fibrosis, resulting in cardiac diastolic dysfunction, systolic dysfunction, and heart failure. Abbreviations: AGEs, advanced glycation end-products; RAAS, renin-angiotensin-aldosterone system.

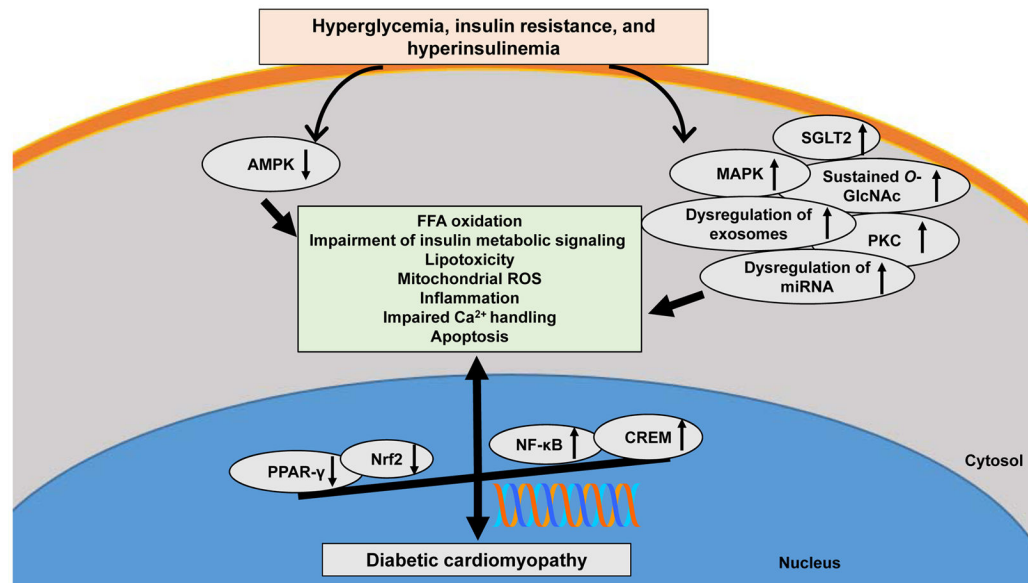


Fig. 2.

The molecular proteins and signaling pathways in hyperglycemia- and insulin resistance-diabetic cardiomyopathy. Increased PKC, MAPK, NF- κ B, SGLT2, *O*-GlcNAc and CREM signaling, dysregulation of miRNA and exosomes, and reduction of AMPK, PPAR- γ and Nrf2 induce cardiac insulin resistance, subcellular component abnormalities, metabolic disorders, and structural changes, resulting in diabetic cardiomyopathy. Abbreviations: AMPK, AMP-activated protein kinase; PPAR, peroxisome proliferator-activated receptor; Nrf2, nuclear factor erythroid 2-related factor 2; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; NF κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; SGLT2, sodium-glucose cotransporter-2; *O*-GlcNAc, *O*-linked N-acetylglucosamine; CREM, cyclic adenosine 5'-monophosphate-responsive element modulator; miRNA; microRNA.