Predictors and prevention of diabetic cardiomyopathy

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Abstract: Despite our cognizance that diabetes can enhance the chances of heart failure, causes multiorgan failure, and contributes to morbidity and mortality, it is rapidly increasing menace worldwide. Less attention has been paid to alert prediabetics through determining the comprehensive predictors of diabetic cardiomyopathy (DCM) and ameliorating DCM using novel approaches. DCM is recognized as asymptomatic progressing structural and functional remodeling in the heart of diabetics, in the absence of coronary atherosclerosis and hypertension. The three major stages of DCM are: (1) early stage, where cellular and metabolic changes occur without obvious systolic dysfunction; (2) middle stage, which is characterized by increased apoptosis, a slight increase in left ventricular size, and diastolic dysfunction and where ejection fraction (EF) is <50%; and (3) late stage, which is characterized by alteration in microvasculature compliance, an increase in left ventricular size, and a decrease in cardiac performance leading to heart failure. Recent investigations have revealed that DCM is multifactorial in nature and cellular, molecular, and metabolic perturbations predisposed and contributed to DCM. Differential expression of microRNA (miRNA), signaling molecules involved in glucose metabolism, hyperlipidemia, advanced glycogen end products, cardiac extracellular matrix remodeling, and alteration in survival and differentiation of resident cardiac stem cells are manifested in DCM. A sedentary lifestyle and high fat diet causes obesity and this leads to type 2 diabetes and DCM. However, exercise training improves insulin sensitivity, contractility of cardiomyocytes, and cardiac performance in type 2 diabetes. These findings provide new clues to diagnose and mitigate DCM. This review embodies developments in the field of DCM with the aim of elucidating the future perspectives of predictors and prevention of DCM.

Keywords: diabetes, obesity, exercise, heart failure, miRNA, oxidative stress

Introduction
Diabetes mellitus (DM) is a metabolic disorder with multiple etiology and is one of the leading causes of morbidity and mortality worldwide.1–3 The prevalence of DM is increasing at an alarming rate and is predicted to occur in approximately 5% of the global population by 2025.4,5 There are two major types of DM: (a) type 1 diabetes (T1D) which is caused by deficiency or absence of insulin due to destruction of pancreatic beta cells and (b) type 2 diabetes (T2D) which is characterized by insulin insensitivity or intolerance. T1D is prevalent in the young (also called juvenile diabetes) and T2D is prevalent in adults.6 However, the number of both T1D and T2D patients is expected to increase 3–4 fold by 2050 in the United States.7 The progression from prediabetes to diabetes has also contributed to the rapid increase in the number of people diagnosed with diabetes. It is estimated that nearly 5%–10% of the global population per year
either progress to diabetes or improve reverting to normal glucose levels. However, the size of the prediabetic population is increasing worldwide and it is estimated that nearly 470 million people will have prediabetes by 2030.8

DM is associated with both microvascular (including retinopathy, nephropathy, and neuropathy) and macrovascular (including cardiovascular diseases) complications.9-12 Clinical studies suggest that the incidence of heart failure is 2-4 fold higher in diabetics when compared to nondiabetic patients.13,14 Diabetic cardiomyopathy (DCM) is described as the structural and functional changes in the myocardium that are associated with diabetes in the absence of ischemic heart diseases, hypertension, or other cardiac pathologies.2,3,15-19 Although it has been four decades since DCM was described, the pathogenesis and underlying mechanisms of the disease are not completely understood. Glucose has been considered as the main driving force for the development of DCM,16 however, recent clinical trials (UK Prospective Diabetes Study 33[UKPDS33], the Action to Control Cardiovascular Risk in Diabetes [ACCORD], the Action in Diabetes and Vascular Disease [ADVANCE] and the Veterans' Administration Diabetes [VADT])20 have revealed no significant effect of intensive glycemic control on mortality and amelioration of cardiovascular events.17 Hence, there is a dire need to understand the detailed mechanisms and factors associated with DCM. Additionally, novel approaches such as stem cell therapy, and micro-RNA (miRNA) may be a promising therapeutic target, for the treatment of DCM. This article embodies a brief overview of DCM, its predictors and preventative measures (at different stages of disease), and future perspectives of therapy.

DCM

Early studies have demonstrated that coronary artery disease (CAD) is the primary cause of cardiac death in diabetics.19,21 However, this notion was challenged by findings that there was a modest increase in atherosclerotic disease in diabetics when compared with age- and sex-matched nondiabetic controls,22 and absence of narrowing of the lumen in the intramuscular vessels in diabetics.23 These findings were enigmatic until 1972 when DCM was identified as heart failure without any clear symptom of hypertension, CAD, or valvular disease.24 DCM was corroborated by examination of left ventricular function and coronary angiogram in uncomplicated adult diabetics with a family history,25 where a significant reduction of stroke volume index and an elevation in end-diastolic pressure were demonstrated in diabetics when compared with age-matched controls. Although no difference in ejection fraction (EF) was recorded between diabetics and control, there was a significant increase in end-diastolic filling pressure to volume (indicator of end-diastolic wall stiffness) in diabetics.26 Based on these findings, DCM is defined as a distinct entity characterized by the presence of abnormal myocardial performance or structure in the absence of epicardial CAD, hypertension, and significant valvular disease.15,20 Due to the multifactorial nature of diabetes, there are perturbations at both the cellular and molecular levels that predispose the heart to pathological structural and functional remodeling. These alterations may contribute to DCM; however, the detailed mechanism is not completely understood. Cardiomyopathy can be classified into two types: (1) primary cardiomyopathy where the cardiomyopathy primarily affects the function of the heart and (2) secondary cardiomyopathy where cardiac performance is affected due to a systemic syndrome.27 Cardiomyopathy leads to heart failure which can be either systolic heart failure with reduced EF or diastolic heart failure with normal EF.28 The definition of DCM has been extended to DCM with normal EF and DCM with reduced EF, and includes all associated diabetic diseases affecting central hemodynamics.16

Risk factors for DCM

As diabetes is associated with DCM, elevated glucose level seems to be the major risk factor.16 However, the risk factors that contribute to diabetes or heart failure are also associated with DCM. These risk factors include a high fat diet-obesity, cardiovascular autonomic neuropathy (CAN), inflammation and elevated levels of free fatty acid (FFA), advanced glycation end products (AGEs) and their receptors, and reactive oxygen species (ROS). Recently, differential expression of miRNAs29,30 and stem cell survival and differentiation31 was associated with DCM (Figure 1).

Mechanism underlying DCM

The disturbances in metabolism that lead to hyperlipidemia, insulin insensitivity causing hyperinsulinemia, and deficiency of insulin due to pancreatic beta cell death causing hyperglycemia, contribute to DCM.1 The metabolic disturbances is mainly due to an elevation in nonesterified fatty acids, also called free fatty acid (FFA).2,3,16 The heart has the potential to utilize both FFA and carbohydrate as a source of energy. However, the dominant source of energy is FFA, and this switches to carbohydrate with increased workload or starvation.32 The switch from FFA to carbohydrate may be due to fetal gene reprogramming.33 In the heart of diabetics, energy production by glucose utilization may be decreased and FFA utilization is increased and this causes depletion of glucose transporter (GLUT)-1 and -4.34 The transgenic expression of GLUT-4 in diabetic mice restored cardiac metabolism and function, and this suggested that glucose metabolism
was associated with DCM.\textsuperscript{35} The elevated level of FFA is implicated in cellular insulin resistance.\textsuperscript{1,14} One mechanism by which FFA induces insulin resistance is through the protein kinase C (PKC) pathway. PKC (a serine/threonine kinase) phosphorylates inhibitor of kappa light polypeptide gene enhancers B-cells (IkKB) kinase, and this in turn phosphorylates insulin receptor substrate-1 (IRS-1). Phosphorylation of IRS-1 inhibits its ability to bind to the p85 regulatory subunit of phosphatidylinositol 3-kinase, impairing insulin signal transduction in skeletal muscle.\textsuperscript{36} However, this signaling cascade is not demonstrated in the diabetic heart. Another mechanism of FFA-mediated insulin resistance is via peroxisome proliferator-activated receptor (PPAR)-\(\gamma\). The activation of PPAR-\(\gamma\) is associated with increased FFA that in turn induces the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN). PTEN dephosphorylates phosphatidylinositol-3, 4, 5-triphosphate and this prevents activation of Akt (serine/threonine kinase)-1.\textsuperscript{3,37} Increased levels of insulin induces cardiac hypertrophy by inhibiting glycogen synthase kinase-3\(\beta\), which inhibits nuclear transcription of the hypertrophic program through nuclear factor in activated lymphocytes.\textsuperscript{38,39} Elevated levels of insulin also upregulates Akt-1 and this induces mammalian target of rapamycin (mTOR) that in turn activates the p70 ribosomal subunit S6 kinase-1 and promotes protein synthesis contributing to cardiac hypertrophy.\textsuperscript{40–42}

Peroxisome proliferator-activated receptor (PPAR-\(\alpha\)) is also activated by FFA and its activation induces pyruvate dehydrogenase kinase-4 causing glucose oxidation and stimulating fatty acid uptake in the mitochondria. Along with an increase in long chain acyl carnitines, it promotes mitochondrial uncoupling of oxidative phosphorylation.\textsuperscript{43} Mitochondrial uncoupling of oxidative phosphorylation results in decreased myocardial high energy reserves and contractile dysfunction.\textsuperscript{19} The elevated level of FFA abrogates pyruvate dehydrogenase and this induces accumulation of glycolytic intermediates and ceramides, which may promote apoptosis.\textsuperscript{44,45} The lipotoxicity due to toxic metabolites from FFA opens K-ATP channels\textsuperscript{46} and this impairs the ability of cardiomyocytes to regulate calcium use, causing contractile dysfunction.\textsuperscript{47–49} The induction of apoptosis, hypertrophy, and contractile dysfunction leads to DCM (Figure 2A).

Hyperglycemia also triggers ROS\textsuperscript{50–52} by inducing glucose oxidation and generating mitochondrial superoxide.\textsuperscript{52–55} ROS activates matrix metalloproteinases 9 (MMP9) which degrades extracellular matrix, increases matrix turnover, attenuates sarco-endoplasmic reticulum-calcium ATPase 2 (SERCA2), and alters the expression of several miRNAs that leads to contractile dysfunction and ultimately DCM.\textsuperscript{31,55–56}

In diabetes, induction of MMP9 also increases inflammation by inducing pro-inflammatory tumor necrosis factor (TNF)-\(\alpha\) and mitigating the anti-inflammatory interleukin (IL)-10 cytokine (unpublished data, 2013) which exacerbates DCM.\textsuperscript{55–60} Differential expression of several miRNAs also induces TNF-\(\alpha\), inhibits IL-10, and regulates inflammation.\textsuperscript{61–63} Several miRNAs (such as miR-155 and miR-223) are anti-inflammatory and cardioprotective.\textsuperscript{54,65} Diabetes-mediated generation of superoxide also causes DNA damage that triggers the reparative enzyme poly (ADP ribose) polymerase (PARP).\textsuperscript{50} The induction of PARP attenuates glyceraldehyde phosphate dehydrogenase and this diverts glucose from the glycolytic pathway into alternative pathways such as increased glycation end products (AGEs) and the PKC pathway which downregulates the calcium regulating receptor and enzyme ryanodine receptor and SERCA2 respectively, impairing the contractility of cardiomyocytes, and inducing the ventricular stiffness that leads to DCM (Figure 2B).\textsuperscript{66–72}

Diabetes cardiovascular autonomic neuropathy is manifested in both T1D and T2D, and is diagnosed with abnormal variation in diurnal and nocturnal blood pressure, resting heart rate disorder, exercise intolerance, and prolongation of QT interval in ECG.\textsuperscript{72} The attenuations of beta-1 and -2 adrenergic receptors (which are part of sympathetic tone) is also associated with DCM.\textsuperscript{74–77} The alterations in myocardial autonomic neurotransmitters cause toxic effects on catecholamines and apoptosis\textsuperscript{79} which contributes to DCM. The microenvironment (oxidative stress, myocardial stiffness, differential expression of miRNAs) of the myocardium is changed in
the heart of diabetics, and this is implicated in defective cardiac progenitor cell growth and differentiation,\textsuperscript{16,31} which contributes to DCM.

**Pathophysiology and remodeling in DCM**

The high fat diet/obesity is associated with insulin resistance, T1D and T2D. In T2D, high blood glucose levels trigger pancreatic beta cells to release insulin; however, due to insulin insensitivity, glucose levels remain high. These hyperglycemic signals continuously activate beta cells to release insulin leading to hyperinsulinemia. The beta cells, due to their continued workload, die and thus, in the long-term, T2D leads to T1D (Figure 3). Diabetes is associated with structural (fibrosis,\textsuperscript{55,78} apoptosis,\textsuperscript{55,79,80} angiopathy,\textsuperscript{81–83}) functional (endothelium–myocytes uncoupling,\textsuperscript{55} impaired

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**Figure 2** (A) Different pathways associated with increased free fatty acid mediated diabetic cardiomyopathy and (B) different pathways associated with hyperglycemia mediated diabetic cardiomyopathy.

**Notes:** “↑” indicates increased levels and “↓” indicates decreased levels.

**Abbreviations:** AGE, advanced glycation end product; AKT-1, serine/threonine kinase; DCM, diabetic cardiomyopathy; ECM, extracellular matrix; FFA, free fatty acid; GAPDH, glyceraldehyde phosphate dehydrogenase; GLUT, glucose transporter; GSK-3β, glycogen synthase kinase-3β; K-ATP, ATP sensitive potassium channel; miRNA, micro-RNA; MMP9, matrix metalloproteinase 9; mTOR, mammalian target of rapamycin; PARP, poly(ADP ribose) polymerase; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA2, sarco-endoplasmic reticulum-calcium ATPase 2; TNF-α, tumor necrosis factor-α.
contractility of cardiomyocytes,

increased survival and differentiation of cardiac stem cells,

diastolic and systolic dysfunction,

and regulatory (alteration in the levels of miRNAs and signaling molecules involved in glucose metabolism) remodeling that leads to DCM (Figure 3).

There are three major stages of DCM: early stage, middle stage, and late stage. The early stage is asymptomatic, where the heart becomes hypertrophic and has diastolic dysfunction with normal EF. However, at the molecular level, increased levels of FFA, altered calcium homeostasis, and depletion of GLUT-1 and -4 are evident. The middle stage is recognized by increased left ventricle (LV) size, wall thickness, and mass, which is accompanied by diastolic dysfunction and a slight decrease in systolic function (EF < 50%). It is also accompanied by insulin resistance, AGE formation, increased levels of renin-angiotensin-aldosterone system (RAAS) and tumor growth factor-β1, reduced levels of insulin growth factor-1, apoptosis, necrosis, fibrosis, and mild CAN.

The progression from middle stage to late stage disease is associated with additional severities including microvascular changes, CAD, and CAN, which impairs both systolic and diastolic functions (Table 1).

**Predictors and prevention of DCM**

The alarming increase in the number of diabetic patients with cardiomyopathy warrants the implementation of diagnostic strategies for DCM to identify the disease at its early stages. Currently, there is no well recognized method for early diagnosis of DCM. DCM induces changes in the heart structure (myocardial hypertrophy, fibrosis, and fat droplet deposition) and early changes in cardiac function are evident by the abnormal diastolic function that progresses to systolic dysfunction in the later stage of disease. These changes in patients with DCM can be diagnosed using the following methods:

**Table 1** The phenotype and functional impairment in different stages of diabetic cardiomyopathy

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cellular mechanism</th>
<th>Structural change</th>
<th>Functional change</th>
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<tbody>
<tr>
<td>Early</td>
<td>Increased FFA; altered Ca&lt;sup&gt;2+&lt;/sup&gt; homeostasis; depleted GLUT-1 and GLUT-4</td>
<td>Slightly increased LV size, wall thickness, and mass</td>
<td>Possible diastolic dysfunction, normal ejection fraction</td>
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<tr>
<td>Middle</td>
<td>Insulin resistance; AGE formation; increased RAAS and TGF-β1; reduced IGF-1; apoptosis; necrosis; fibrosis; mild CAN</td>
<td>Increased LV size, wall thickness, and mass, dilatation, fibrosis</td>
<td>Diastolic dysfunction, ejection fraction is &lt; 50%</td>
</tr>
<tr>
<td>Late</td>
<td>Hypertension; microvascular changes; severe CAN; CAD</td>
<td>Increased LV size, wall thickness, and mass, dilatation, fibrosis, micro-angiopathy</td>
<td>Systolic and diastolic dysfunction</td>
</tr>
</tbody>
</table>

**Abbreviations:** AGE, advanced glycation end product; CAN, cardiovascular autonomic neuropathy; CAD, coronary artery disease; FFA, free fatty acid; GLUT, glucose transporter; IGF-1, insulin growth factor-1; TGF-β1, transforming growth factor-β1; LV, left ventricle; RAAS, renin-angiotensin-aldosterone system.
1. Echocardiography and Doppler imaging: In the early stage of DCM and in the majority (75%) of asymptomatic diabetic patients, diastolic dysfunction characterized with heart failure with normal EF is present. Diastolic dysfunction, mitral inflow patterns, mitral E/E', transmitial E/A, cardiac stiffness, and dilatation of the LV can be assessed by echocardiography. Therefore, echocardiography and Doppler imaging can be utilized to evaluate structural and functional remodeling in the heart of diabetics. However, numerous factors such as myocardial fibrosis, hypertrophy, and contractile asynchrony changes in calcium cycling are involved in altering the normal LV diastolic function. These changes are not confined to DCM but are also present in other cardiac diseases, and therefore, other approaches are required for diagnosing DCM.

2. Magnetic resonance imaging (MRI): MRI is a highly sensitive tool for detecting LV wall motion abnormalities, geometry, and cardiac hypertrophy. MRI is considered to be a favorable tool for the accurate measurement of LV mass, volume, and function. Additionally, it provides information on arrhythmia and cardiomyopathy.

3. Serological biomarkers:
   a. High levels of glucose and hemoglobin A\textsubscript{1c} are indicators of diabetes.
   b. Increased levels of N-terminal pro-brain natriuretic peptide and brain natriuretic peptide are markers of heart failure.
   c. Troponin present in the plasma, is an indicator of necrosis.
   d. Elevated level of MMPs (especially MMP9) and decreased levels of tissue inhibitor of metalloproteinases (TIMPs) are indicators of fibrosis.
   e. Levels of the enzyme beta O-GlcNAc (o-linked N-acetylglucosamine) can also be used as a predictor of DCM as it is increased in hypertrophy and cardiovascular diseases.

4. Heart catheterization and coronary angiography: Different stages of DCM can be diagnosed by left heart catheterization that assesses LV end diastolic pressure and right heart catheterization that measures mean pulmonary wedge pressure which is often associated with increases in mean pulmonary pressure. Coronary angiography determines stenosis in the coronary artery which is often present in late stages of DCM.

MiRNA as a potential biomarker for DCM
MiRNA are small (~22 nucleotide), conserved, non-coding RNA molecules that modulate gene expression either through mRNA degradation or translational repression. They are emerging as promising therapeutic targets for cardiovascular disease and diabetes. The levels of miRNAs are altered in the hearts of diabetics. Recently, circulating miRNAs were reported as biomarkers for cardiovascular disease. Therefore, the differential expression of specific circulating miRNAs can be used to diagnose different stages of DCM (Table 2).

Prevention of DCM
Although tight regulation of glucose levels is thought to ameliorate DCM, recent clinical trials (UKPDS 33, ACCORD, ADVANCE, and VADT) have failed to support this. Based on the different stages of DCM, different preventative measures should be taken.

Early stage DCM: Changes in lifestyle and diet are the measurable factors that prevent DCM progression and may even cure the disease. A low fat and glucose diet, and physical exercise can mitigate early DCM.

Middle stage DCM: In addition to exercise and diet control, treatment with metformin for T2D, insulin for T1D, pioglitazone for ameliorating diastolic dysfunction, and beta-blockers for reducing blood pressure may be required.

Late stage DCM: In addition to the above mentioned preventative measures for middle stage DCM, angioplasty is required to mitigate micro- and macro-angiopathy and for coronary stenosis (Table 2).

Antidiabetic drugs for treatment of DCM
Metformin (one of the most commonly prescribed drugs) improves peripheral sensitivity to insulin, promotes hyperglycemic control, and acts as an anti-inflammatory agent. Glucagon-like peptide (GLP)-1 is an incretin hormone that stimulates postprandial insulin secretion and improves insulin sensitivity. Individuals treated with GLP-1 also have improved left ventricular ejection fraction.

Other classes of drugs for treatment of DCM
The trials with statins and RAAS inhibitors also show positive results for mitigating DCM. Statins inhibit cholesterol biosynthesis, and have anti-inflammatory and anti-oxidative stress functions. They also improve LV function and reduce fibrosis that ameliorates DCM. RAAS inhibitors are also cardioprotective. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are commonly used to block the RAAS. Results based on clinical and experimental studies suggest that RAAS inhibitors not only reduce...
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blood pressure but also help to improve insulin sensitivity and enhance blood glucose uptake. Diastolic dysfunction is ameliorated by RAAS inhibitor treatment.91 The use of beta-blockers in diabetic patients is not common considering different parameters such as blood glucose, insulin resistance, and dyslipidemia; however, recent meta-analyses suggest that the use of these beta-blockers improves glycemic levels and insulin resistance when compared with other antidiabetic drugs.91

Conclusions and future directions
Several hypotheses have been proposed to describe how DCM develops,3,16 however, DCM is still a valid challenge in medical science as the number of diabetics in the population is rapidly increasing. Although recent trials have revealed that tight glucose regulation is not as effective as first thought, control of hyperglycemia is essential to mitigate DCM. It is clear that DCM is regulated not only by high glucose levels; there are several other factors and mechanisms (Figures 1–3) that contribute to DCM. The role of high blood pressure, hyperlipidemia, and oxidative stress also contributes to, and exacerbates, DCM. Early diagnosis is essential for preventing and reverting DCM. For early diagnosis, serological markers, echocardiography, and MRI are important. Recent advances in the areas of miRNA and stem cell therapy provide a new dimension to explore DCM and its therapy. The levels of specific miRNA during early, middle, and late stage DCM can be used as a biomarker for different stages of DCM. The use of miRNA mimics and antagomiR (if a miRNA is attenuated and up regulated, respectively) can be exploited to mitigate DCM. Similarly, stem cells can be used for regenerating pancreatic beta cells and myocardium to improve glucose metabolism and cardiac function, respectively.

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