Conundrum of pathogenesis of diabetic cardiomyopathy: Role of vascular endothelial dysfunction, reactive oxygen species, and mitochondria

ARTICLE *in* MOLECULAR AND CELLULAR BIOCHEMISTRY · DECEMBER 2013

Impact Factor: 2.39 · DOI: 10.1007/s11010-013-1861-x · Source: PubMed

9 AUTHORS, INCLUDING:

Sainath Kotha
The Ohio State University
42 PUBLICATIONS **269** CITATIONS
SEE PROFILE

Vaithinathan Selvaraju
University of Connecticut
37 PUBLICATIONS **395** CITATIONS
SEE PROFILE

Abhay Satoskar
The Ohio State University
211 PUBLICATIONS **7,416** CITATIONS
SEE PROFILE

Narasimham L Parinandi
The Ohio State University
122 PUBLICATIONS **2,842** CITATIONS
SEE PROFILE

Mol Cell Biochem (2014) 386:233–249
DOI 10.1007/s11010-013-1861-x

Conundrum of pathogenesis of diabetic cardiomyopathy: role of vascular endothelial dysfunction, reactive oxygen species, and mitochondria

Mandip Joshi · Sainath R. Kotha · Smitha Malireddy · Vaithinathan Selvaraju · Abhay R. Satoskar · Alexender Palesty · David W. McFadden · Narasimham L. Parinandi · Nilanjana Maulik

Received: 22 July 2013 / Accepted: 9 October 2013 / Published online: 4 December 2013

Abstract Diabetic cardiomyopathy and heart failure have been recognized as the leading causes of mortality among diabetics. Diabetic cardiomyopathy has been characterized primarily by the manifestation of left ventricular dysfunction that is independent of coronary artery disease and hypertension among the patients affected by diabetes mellitus. A complex array of contributing factors including the hypertrophy of left ventricle, alterations of metabolism, microvascular pathology, insulin resistance, fibrosis, apoptotic cell death, and oxidative stress have been implicated in the pathogenesis of diabetic cardiomyopathy. Nevertheless, the exact mechanisms underlying the pathogenesis of diabetic cardiomyopathy are yet to be established. The critical involvement of multifarious factors including the vascular endothelial dysfunction, microangiopathy, reactive oxygen species (ROS), oxidative stress, mitochondrial dysfunction has been identified in the mechanism of pathogenesis of diabetic cardiomyopathy. Although it is difficult to establish how each factor contributes to disease, the involvement of ROS and mitochondrial dysfunction are emerging as front-runners in the mechanism of pathogenesis of diabetic cardiomyopathy. This review highlights the role of vascular endothelial dysfunction, ROS, oxidative stress, and mitochondriopathy in the pathogenesis of diabetic cardiomyopathy. Furthermore, the review emphasizes that the puzzle has to be solved to firmly establish the mitochondrial and/or ROS mechanism(s) by identifying their most critical molecular players involved at both spatial and temporal levels in diabetic cardiomyopathy as targets for specific and effective pharmacological/therapeutic interventions.

Keywords Diabetic cardiomyopathy · Hyperglycemia · Vascular endothelial dysfunction · Reactive oxygen species · Cardiac mitochondriopathy

M. Joshi · V. Selvaraju · D. W. McFadden · N. Maulik (✉)
Department of Surgery, University of Connecticut Health Center, Farmington Avenue, Farmington, CT 06032, USA
e-mail: nmaulik@neuron.uchc.edu

M. Joshi · A. Palesty
Department of Surgery, Saint Mary's Hospital, Waterbury, CT, USA

S. R. Kotha · S. Malireddy · N. L. Parinandi
Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, Dorothy M. Davis Heart & Lung Research Institute, The Ohio State University College of Medicine, Columbus, OH, USA

A. R. Satoskar
Department of Pathology, The Ohio State University College of Medicine, Columbus, OH, USA

Introduction

Diabetes is one of the leading causes of death and disability throughout the world. It is associated with blindness, strokes, kidney failure, and vascular, heart and nerve diseases.

Diabetes in epidemic proportions worldwide

According to the report released by the International Diabetes Federation in 2011, 366 million people worldwide are affected by diabetes, and that number is estimated to rise to 552 million by 2030. Between 2010 and 2030, there will be a 69 % increase in the number of adults with diabetes in

developing countries and a 20 % increase in the developed countries [1]. The global health expenditure on diabetes was estimated to account for a total of at least US $376 billion in 2010 and is expected to reach US $490 billion in 2030. Worldwide, approximately 12 % of the healthcare expenditure (US $1330/person) was allocated to diabetes care in 2010 [2].

Salient features of diabetic cardiomyopathy

The relationship between diabetes and heart disease is well established from several animal and human studies [3–5]. The term "Diabetic Cardiomyopathy" was first coined by Rubler 30 years ago [6]. Diabetic cardiomyopathy refers to structural changes in the heart, such as chamber enlargement, increased fibrosis and left ventricular (LV) mass [7]. In the Framingham study conducted on an unselected cohort of 5,209 patients, men between 45 and 74 years of age exhibited more than twice the frequency of congestive heart failure as opposed to their non-diabetic cohorts, and diabetic women showed a fivefold increased risk of congestive heart failure (CHF). Furthermore, the correlation between heart failure (HF) and diabetes still persisted even after taking age, blood pressure, weight, cholesterol levels, and coronary heart disease into account [8]. In a study conducted on 2,737 patients (mean age of 81 years) without HF and with and without diabetes, 39 % of the diabetic subjects developed CHF as compared to 23 % of subjects in the non-diabetic group with a relative risk of 1.3 [9]. Another retrospective cohort study conducted on 17,076 subjects (8,231 patients with type 2 diabetes and 8,845 non-diabetic patients) revealed 30.9 % incidence of CHF in the diabetic group as compared to 12 % of incidence of CHF in the non-diabetic group, with a relative risk of greater than 2.5 [10]. These studies are supported by United Kingdom Prospective Diabetes Study (UKPDS), which has revealed that the prevalence of HF decreases with the decrease in blood sugar, as measured by serum hemoglobin A1c (HbA1c) [11]. Understanding the relationship between hyperglycemia and CHF is vital in combatting diabetes since cardiogenic and related complications are the leading causes of morbidity and mortality in these individuals [12]. The STENO 2 study has demonstrated that the cardiovascular mortality in diabetic patients remains high in spite of intensive treatment of all the associated cardiovascular risk factors, treatments that decreased the incidence of cardiovascular events by 50 % [13].

The incidence of diabetic cardiomyopathy in type 1 versus type 2 is variable between different clinical and human studies. However, in humans in the long run, the incidence looks quite similar in both the types. Regarding the pathogenesis, it almost is similar in humans with type1 diabetes compared to type 2, because despite being type 1, well-controlled individuals receive exogenous insulin, thus are not hypoinsulinemic. Systolic dysfunction in type 1 diabetes in humans, is less evident than in STZ-induced models because of the exogenous insulin, making them metabolically akin to a type 2 diabetic [14].

Early changes in the heart during diabetes are characterized by abnormal diastolic function, ultimate loss of systolic function, and overt clinical symptoms [15, 16]. The pathogenesis of diabetic cardiomyopathy broadly involves hyperglycemia, lipotoxicity, and insulin resistance contributing to reactive oxygen species (ROS) generation, mitochondrial dysfunction, impaired calcium metabolism, Renin–Angiotensin System (RAS) activation, altered substrate metabolism, and endothelial dysfunction, which ultimately lead to diastolic dysfunction and HF (Fig. 1).

In this review we present the putative mechanisms operated by endothelial dysfunction, involvement of ROS, and mitochondrial dysfunction and current therapeutic strategies/options of diabetic cardiomyopathy.

Functional changes of myocardium during diabetic cardiomyopathy

Echocardiography is an extremely useful, inexpensive tool for the researcher and to clinician to assess the cardiac function in patients and in animal models. Transmitral Doppler imaging is commonly used for measuring the diastolic function in the heart [17]. The four useful variables from mitral flow measurement are the (i) early peak diastolic transmitral flow velocity (*E*), (ii) late peak diastolic transmitral flow velocity (*A*), (iii) A-wave duration (Adur), and (iv) early filing deceleration time (DT). These values vary with the severity of the disease [18]. Tissue Doppler Imaging (TDI) echocardiography has emerged as a very sensitive and effective technique in recent years for the assessment of diastolic function [19]. Several studies have shown that TDI is superior to conventional flow echocardiography, but the combination of both techniques enhances the ability to diagnose diastolic dysfunction at early stages in diabetics [20, 21].

The association of diabetes with diastolic dysfunction has been well established [15, 20, 22–25]. The Hoorn study has shown that Diabetes Mellitus type 2 (DM2) is independently associated with a 2.0-fold greater risk for systolic dysfunction and a 2.4-fold greater risk for diastolic dysfunction [26]. Diastolic dysfunction usually precedes the systolic dysfunction [7, 15, 23]. A population-based cohort study of more than 2,000 patients has shown an increased prevalence of diastolic dysfunction and a tendency of the diastolic dysfunction to progressively worsen with advancing age [27]. Studies have shown that the diastolic dysfunction is more common in type 2 diabetics

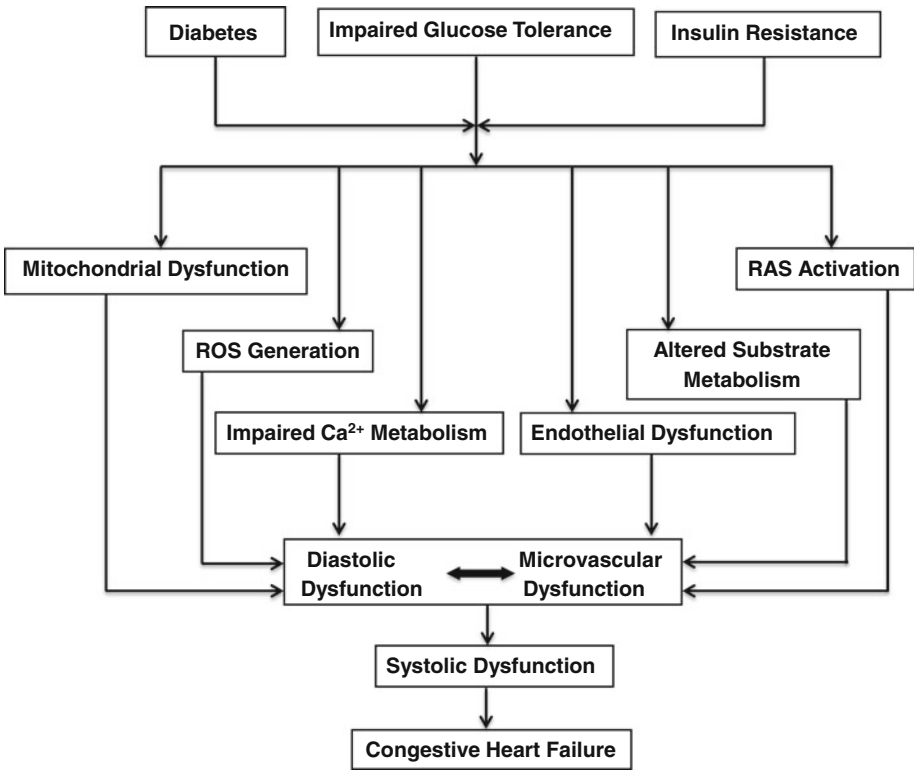

Fig. 1 Mechanisms of pathogenesis of diabetic cardiomyopathy. The pathogenesis of diabetic cardiomyopathy broadly involves hyperglycemia, lipotoxicity, and insulin resistance contributing to reactive oxygen species (ROS) generation, mitochondrial dysfunction, impaired calcium metabolism, RAS activation, altered substrate metabolism, and endothelial dysfunction which ultimately lead to diastolic dysfunction and heart failure

than in type 1 diabetes in the earlier stages of the disease without overt cardiovascular symptoms [28, 29]. However, not all studies have shown association of diabetes with diastolic dysfunction. A study conducted on children (4–20 years of age) consisting of 61 diabetic and 23 non-diabetic subjects failed to show any association between the disease and the studied variables pertinent to LV function [30].

Metabolic syndrome and alterations in myocardial functions

The metabolic syndrome that coexists with diabetes augments LV diastolic dysfunction (LVDD), both in prevalence and severity [31]. Studies have reported the presence of LVDD in patients before diagnosis of overt diabetes mellitus suggesting that LVDD develops temporally with impaired glucose tolerance and insulin resistance. The study involved 208 patients, and the patients with insulin resistance showed significantly higher association with LVDD as compared to the non-insulin resistant group [32]. In similar studies, the patients with impaired glucose tolerance test exhibited diastolic dysfunction as high as 50–74 % [33, 34]. Screening these populations with diastolic dysfunction at earlier stages may lower the risk of HF, thereby alleviating financial burden to the patient and to society. Systolic function also serves as a reliable marker for diabetic cardiomyopathy as evidenced by lower peak strain, strain rates, and cyclic variation indexes of the septum and posterior [35], lower systolic and diastolic function reserve indices [36], impaired radial and longitudinal LV systolic function, [37], and left atrial electromechanical delay [38].

Myocardial alterations in animal models of diabetes

In various animal models of diabetes, the functional and pathophysiological changes seen in human studies also have been documented [39]. Several models of diabetes (Types 1 and 2) have been developed and studied both in vivo, ex vivo (e.g., isolated perfused heart), and in vitro (e.g., cardiomyocytes). Most of these studies have shown decreased systolic and diastolic functions during diabetes in both in vivo and ex vivo models. Increased LV mass and LV stiffness were established in several studies with animal models [40–44]. A study performed on 24-week-old db/db mouse (type 2 diabetes model) found decreased LV contractility but normal ejection fraction, cardiac output, and dP/dt [45]. Another study that compared both type 1 diabetes (streptozotocin-induced, STZ) and type 2 diabetes (Zucker diabetic fatty rat, ZDF) showed that the type 1 diabetics exhibited a greater magnitude of systolic dysfunction than diastolic dysfunction while the type 2 diabetics predominantly exhibited diastolic dysfunction with

preserved systolic function [46]. Even though animal models may represent the human disease pattern, their utility is limited due to the inability to induce coronary atherosclerosis in rodents and to tightly control the blood sugar level in animals [47].

Structural changes of myocardium during diabetic cardiomyopathy

Several studies on human subjects and animal models have been carried out to associate myocardial structural changes with the progression of diabetes [48, 49]. A study performed on 145 patients undergoing coronary artery bypass grafting (CABG), some of whom had a history of diabetes revealed increased myocardial hypertrophy, interstitial fibrosis, and capillary endothelial swelling and degeneration in the biopsy specimens of the diabetic heart. Ultrastructural examinations of the tissue samples elucidated capillary basal laminar thickening [49]. Similar examinations on young type I diabetics without cardiovascular disease (CVD) revealed no significant changes in the basal lamina [50], suggesting that the absence of those changes may be related to the duration of the disease and the presence (abundance) of the insulin receptors. If diabetes coexists with hypertension, the pathological changes observed in myocardium including thickening of the capillary basement membrane, interstitial fibrosis, and cell atrophy are amplified [51].

In type 1diabetes, loss of myofibrils, transverse tubules, and sarcoplasmic reticulum was observed 12 weeks after the induction of the disease. Separation of the fasciae adherens at the intercalated disk also was observed and most of these alterations were reversed by insulin administration for 6–12 weeks [52]. Advanced glycation end products (AGEs), which are the metabolic end products of the non-enzymatic glycation, have been linked with the pathogenesis of diabetic cardiomyopathy. In diabetics, AGEs covalently crosslink and alter the structure and function of many proteins, including collagen thereby leading to the development of myocardial fibrosis and stiffness [53–55]. From a study on the type I diabetic rat model, it has been suggested that AGEs play a pivotal role in the pathogenesis of diabetic cardiomyopathy and the cleavage of AGEs with crosslink breaker ALT-711 slows down the process of diabetes associated with the cardiac abnormalities [54]. A study conducted on both diabetic HF patients and a diabetic animal model has shown statistically significant intramyocardial lipid overload and its association with contractile dysfunction [56]. Similarly the alterations in gene expression in ZDF rat hearts have been observed to be similar to those in the failing human heart with lipotoxicity.

Mechanisms of pathogenesis of diabetic cardiomyopathy

Altered calcium metabolism in diabetes and relevance to diabetic cardiomyopathy

Under normal physiologic conditions, action potentials depolarize the cardiomyocyte and open the L-type calcium channel located in the sarcolemma [57]. Entry of calcium ions triggers the release of calcium stored in the sarcoplasmic reticulum through ryanodine receptors (calcium-induced calcium release, CICR) [58]. Free calcium then binds to troponin C, which causes conformational changes of the regulatory complexes leading ultimately to muscle contraction. A small amount of calcium also is pumped out of cytosol by the sodium-calcium exchanger pump, Sarco (Endo) plasmic Reticulum Calcium-ATPase (SERCA) and the mitochondrial calcium uniporter. Phospholamban is an endogenous inhibitor of SERCA, which upon phosphorylation by protein kinases (PKA) gets inactivated and loses its inhibitory effect on SERCA. This leads to decreased levels of calcium in the cytosol and increased calcium levels in the sarcoplasmic reticulum, which allows for faster twitch relaxation [59].

In diabetes, a defect in calcium handling has been proposed as one of the major mechanisms of contractile dysfunction as revealed by several animal model and human studies [60–62]. Proposed causes of pathology include (i) altered SERCA activity and (ii) altered SR calcium storage and defects in ryanodine receptors [63]. Studies conducted on the animal models have shown that the diabetic heart exhibits decreased SERCA activity [64, 65]. Decreased SERCA activity has been linked with decreased expression and function of SERCA and increased inhibition of SERCA activity by overexpression of phospholamban in diabetes. All these changes have been shown reversible by insulin replacement [66, 67]. The decreased activity of SERCA in diabetes is thought to arise from the interaction of AGEs with SERCA [68]. Overexpression of SERCA in the transgenic models has been shown to protect the diabetic heart against severe contractile dysfunction [69]. Expression and function of the ryanodine receptors involved in calcium release from the SR also appear decreased during diabetes [66, 68]. However, there are studies, which failed to show any such changes [70]. Decreased level of FK506-binding protein (FKBP 12.6), an accessory protein and stabilizer of the ryanodine receptor, is also presumed to be involved in the HF during diabetes [71]. Furthermore, activity and expression of the sodium-calcium exchanger that contributes to 28 % of calcium removal has been shown to be lower during diabetes [59, 60]. Thus, it can be concluded that defects in intracellular calcium cycling/signaling caused by alterations in function

and expression of the proteins that handle calcium homeostasis lead to cardiomyopathy in diabetics and can be normalized by specific therapeutic interventions.

Endothelial dysfunction in diabetes and association with diabetic cardiomyopathy

Vascular endothelial cells (ECs) play a pivotal role in the maintenance of cardiovascular homeostasis [72]. ECs form the inner lining of blood vessels that separates the circulating blood from the underlying vascular smooth muscle cells. Normal and healthy ECs produce various vasodilators such as nitric oxide, prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor, all of which inhibit platelet aggregation and fibrinolysis, and maintain vascular tone and permeability. ECs are also involved in the production of vasoconstrictors such as endothelin and angiotensin II [73]. The opposing effects of these dilators and constrictors together play an important role in maintaining the coronary vascular structure. The endothelium functions as a semipermeable tissue-barrier that regulates the flow of nutrients and macromolecules. Exposure to high levels of glucose under diabetic conditions can damage the physiological properties of endothelium and alter its physiological processes, which causes enhanced permeability, leukocyte adhesion, and reduced fibrinolysis [74–76].

Dysfunction of endothelium is considered to be one of the early markers in the development of diabetic atherosclerosis. Physiologically, nitric oxide plays a pivotal role in endothelium-dependent vasodilation and blood pressure regulation [77]. Endogenous nitric oxide is produced by the conversion of the amino acid, L-arginine to L-citrulline by the enzyme, nitric oxide synthase (NOS). Of the various isoforms of NOS, NOS III is considered to be important for maintaining vascular tone [78, 79]. Nitric oxide produced in the endothelium by NOS III diffuses into the vascular smooth muscle cells and activates cyclic GMP, thereby relaxing vascular smooth muscles and leading to vasodilation. However, under diabetic conditions, vascular production of free radicals such as superoxide anions can inactivate nitric oxide or reduce its tissue bioavailability thus promoting atherosclerosis [80]. Increased levels of plasminogen activator inhibitor-1 have been observed during insulin-resistant conditions and have been demonstrated to play a key role in the generation and progression of atherosclerosis [81].

Experimental evidence shows that hyperglycemia-induced activation of protein kinase C (PKC) signaling pathway promotes EC layer permeability. Activation of PKC has been shown to reduce the expression of endothelial NOS and NO production in aortic cells [82]. In addition, inflammatory cytokines are also known to play an important role in endothelial dysfunction. Conditions of insulin resistance caused by type 2 diabetes, atherosclerosis, and endothelial dysfunctions are all known to induce the expression of the pro-inflammatory cytokine, TNF-α, which can increase the expression of vascular and intercellular cell adhesion molecules and promote adherence of monocytes [83]. Tumor Necrosis Factor (TNF) also reduces eNOS expression and interferes with NO production (Fig. 2). Moreover, under hyperglycemic conditions, the coronary circulation gets exposed to increasing amounts of acetylcholine, which paradoxically constricts the coronary arteries, thereby leading to coronary vasospasm [84].

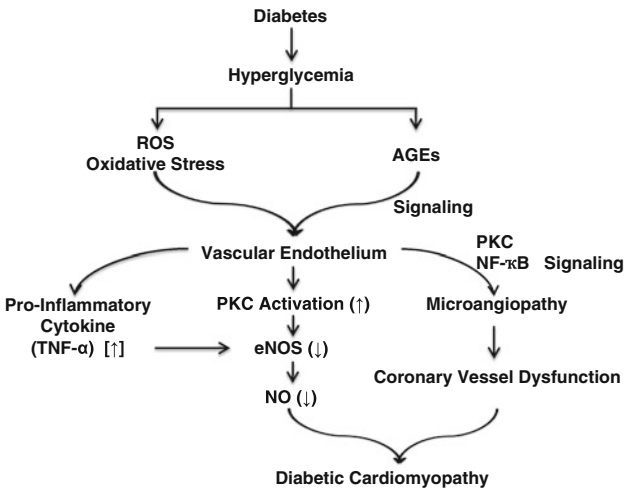

Fig. 2 Vascular endothelial dysfunction in pathogenesis of diabetic cardiomyopathy. Vascular endothelium, the inner monolayer lining of cells surrounding the lumen of the blood vessel acts as a semipermeable barrier and maintains homeostasis of the circulation. Endothelial cells are crucial for both vasodilation and vasoconstriction. Endothelial nitric oxide synthase (eNOS) generates nitric oxide (NO) that is critical for the vascular smooth muscle cell function in vasodilation. Hyperglycemic conditions during diabetes alters the vascular endothelial cell signaling (e.g., decreasing the activity of eNOS through the activation of protein kinase C, PKC) and functions leading to the endothelial dysfunction that is responsible for diabetic microangiopathy and cardiomyopathy

Endothelial dysfunction and related microangiopathy have been linked to the pathogenesis of diabetic cardiomyopathy and HF via cellular signaling cascades involving PKC and nuclear factor-κB (NF-κB) [85]. In this scenario, microvessels undergo diabetes-induced injury causing subsequent destruction of the coronary vasculature. ROS and oxidative stress appear to play a major role in diabetic microangiopathy and the dysfunction of coronary vessels in a hyperglycemia-dependent manner. Hyperglycemia-induced vascular endothelial damage/dysfunction operated through oxidative stress and cell signaling pathways has been identified in the onset and progression of diabetic cardiomyopathy [86]. In the rat model of streptozotocin-diabetes, it has been shown that diabetes induces alterations

in Ca^{2+} homeostasis, SERCA, and sodium–calcium exchanger in cardiac ECs along with diabetes-induced myocardial fibrosis, suggesting that cardiac endothelial alterations play a role in diabetic cardiomyopathy [87]. Transplantation of bone marrow-derived endothelial progenitor cells (EPCs) through intravenous delivery into the streptozotocin-induced diabetic rats has been shown to protect against diabetes-induced myocardial dysfunction, apoptosis of cardiomyocytes, and fibrosis of the heart [88]. This study not only underscores the importance of ECs but also demonstrates the therapeutic use of EPCs in protecting against diabetic cardiomyopathy.

Several therapeutic measures have been shown to yield promising results in improving endothelial dysfunction. Supplementations with the antioxidants such as vitamins C and E, L-arginine, and magnesium have been shown to suppress ROS and induce NO production [89–91]. Drugs that increase insulin sensitivity have also been shown to improve the function of ECs [92, 93]. Non-pharmacological measures such as weight reduction, exercise, and reduced salt intake are also suggested for the recovery of EC functions [94]. One of the intriguing and promising approaches is the use of EPCs in the treatment/protection of diabetic cardiomyopathy [88].

ROS and oxidative stress in diabetic cardiomyopathy

Molecular oxygen, one of the main fuels for energy generation in aerobic organisms, is both a friend and foe. Oxygen undergoes one electron reduction through either enzymatic or non-enzymatic mechanisms to form the superoxide radical, which in turn is converted into hydrogen peroxide by dismutation mediated by the enzyme, superoxide dismutase (SOD) [95]. Enzymes including xanthine oxidase, NAD[P]H oxidase (NOX), and the constituents of the mitochondrial electron transport system are known to activate oxygen to form highly reactive oxygen radicals through the generation of superoxide radical [95]. Hydrogen peroxide reacts with redox-active transition metals including iron (Fe^{2+}) to form the highly reactive radicals [96]. Iron in the biological systems can also be converted into highly reactive ferryl species. Reactive oxygen metabolites such as superoxide radical, hydrogen peroxide, hydroxyl radical, and perferryl/oxoferryl species are collectively called "reactive oxygen species" (ROS) [96]. ROS are known to cause oxidative stress through oxidation of critical biomolecules including proteins, nucleic acids (RNA and DNA), and lipids leading to the damage and dysregulation of the cellular structural, physiological, and metabolic machinery that ultimately causes pathophysiological alterations in the cells, tissues, organs, and the entire organism [97]. Polyunsaturated fatty acids (PUFA) of membrane phospholipids in the living cells are vulnerable to attack of ROS resulting in lipid peroxidation [98, 99]. Lipid peroxidation causes the formation of highly reactive lipid hydroperoxides and reactive carbonyls, which leads to the dysfunction of cellular structure and functions. However, living cells also possess antioxidant defense mechanisms, including enzymatic and non-enzymatic processes aided by (i) the antioxidant enzymes such as SOD (which dismutase superoxide anion), catalase (which removes hydrogen peroxide), and glutathione peroxidase (which converts reactive PUFA hydroperoxides into PUFA hydroxyl species) and (ii) non-enzymatic antioxidant molecules such as glutathione (GSH), vitamin C (ascorbic acid), vitamin E (tocopherol), and myriad dietary antioxidants of plant origin [100–102].

A delicate balance between the extent of production of detrimental ROS and the status of protective antioxidant defense mechanisms in the living cell is essential and critical for the homeostasis of physiological and metabolic functions under normal physiological conditions. Either an overwhelming production of ROS and/or depletion or dysfunction of the antioxidant defense system in the cells is known to cause pathophysiological states including CVDs, cerebrovascular diseases (stroke), neurological diseases, metabolic disorders (e.g., diabetes), lung diseases, and respiratory disorders [103–105]. Strategies of suppression of deleterious actions of ROS, alleviation of ROS-induced oxidative stress, and enhancement of antioxidant status in cells, tissues, and organs by pharmacological treatments or dietary supplementation with antioxidants have been emerging as promising options to combat the ROS and oxidative stress-mediated pathophysiological states and diseases in animal models and humans.

HF and cardiomyopathy have been identified as the foremost causes of mortality among diabetics [106]. Although diabetic cardiomyopathy has been characterized primarily by the manifestation of LV dysfunction among patients affected by diabetes mellitus, a complex array of contributing factors including LV hypertrophy, alterations of metabolism, microvascular pathology, insulin resistance, fibrosis, apoptotic cell death, and oxidative stress have been implicated in the pathogenesis of diabetic cardiomyopathy [106–108]. Nevertheless, the exact mechanisms underlying the pathogenesis of diabetic cardiomyopathy are yet to be established [106]. The critical involvement of ROS and oxidative stress in HF and different types of cardiomyopathy has been delineated, and the importance of the inhibition of xanthine oxidase may attenuate superoxide radical formation and protect against myocardial injury [109]. ROS have been unequivocally established to cause oxidative stress leading to altered cell signaling, apoptosis, and modified gene expression that could lead to diabetic cardiomyopathy. Also, the involvement of ROS and oxidative stress in diabetic CVDs has been emphasized

[110, 111]. Of all the possible mechanisms put forth to describe the pathogenesis of diabetic cardiomyopathy, experimental evidences are mounting for the role of ROS-mediated oxidative stress in the onset and/or progression of diabetic cardiomyopathy [108, 112]. The association of oxidative stress with cardiac damage during diabetes has been recognized [113]. Myocardial disease states including diabetic cardiomyopathy have been shown to be associated with ROS actions and oxidative stress [114]. The involvement of ROS in insulin resistant cardiomyopathy also has been reported [115].

The connection between a lipid-rich diet (high fat) and diabetic cardiomyopathy has been emphasized as the fatty acids take precedence over glucose in uptake and metabolic utilization by the myocardium, which furthers insulin resistance in the cardiac tissue [116]. Fatty acids derived from the fatty diets have been observed to cause elevated state of β-oxidation in cardiomyocyte mitochondria through involvement of the peroxisome proliferator-activated receptors (PPARs), thus causing a metabolic switch in the heart for metabolic utilization of the fatty acids over glucose. This substrate switch has been implicated as a critical factor in the altered cardiomyocyte metabolism leading to the pathogenesis of cardiomyopathy [116]. In addition, the high-fat diet causing fatty heart (accumulation of fat in the myocardium), the condition has also been known to cause overwhelming generation of ROS, which is expected to cause altered insulin signaling cascade and associated alterations in the physiological functions of the heart such as contraction [116]. Therefore, it is highly conceivable that the fat-mediated metabolic switch, typical of the type 2 diabetic state is associated with diabetic cardiomyopathy, wherein the ROS play an important role (Fig. 3). Hyperglycemia leads to the elevated generation of ROS that causes the oxidative stress-induced myocardial damage leading subsequently to the altered gene expression and cellular signal transduction, cardiac cell death, and eventually diabetic cardiomyopathy [117]. Although diabetic cardiomyopathy has been observed to manifest without the involvement of vascular diseases/disorders, ROS-mediated oxidative myocardial injury is gaining precedence over several other plausible mechanisms [117]. Altogether, the hyperglycemic state during diabetes has been marked as the culprit in causing ROS generation and oxidant-induced myocardial damage, which are critical players in the pathogenesis of diabetic cardiomyopathy (Fig. 3).

A case study conducted on a 60-year-old female patient with diabetes, CHF, and hypertrophic cardiomyopathy has revealed a mitochondrial transition mutation (A3243G) [118]. In this study, the electron microscopy of an endocardial biopsy demonstrated proliferation and swelling of mitochondria and induction of heme oxygenase-1 (HO-1)

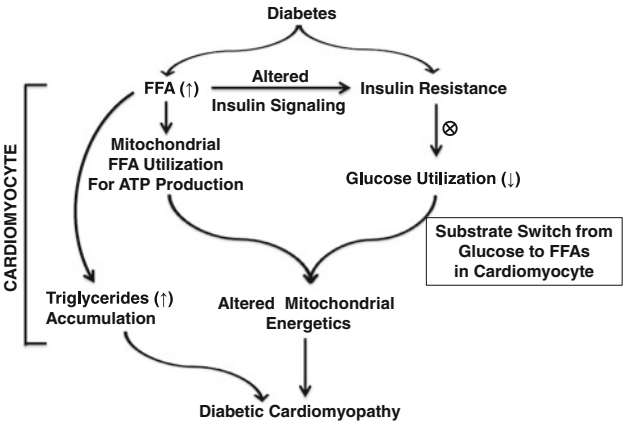

Fig. 3 Substrate switch in pathogenesis of diabetic cardiomyopathy. Elevated levels of free fatty acids (FFAs) are encountered during diabetes in plasma and tissues. Myocardial accumulation of triglycerides (fatty heart) is also known during hyperglycemic conditions. FFAs are preferentially taken up by cardiomyocytes as glucose is not transported into the cell due to either lack of insulin or dysfunction of insulin receptors. FFAs are also known to cause the dysregulation of insulin receptor signaling and insulin resistance through lipotoxicity. Cardiomyocyte mitochondria utilize abundant FFAs as energy substrate by switching the substrate from glucose, which leads to altered mitochondrial energetics and ultimately diabetic cardiomyopathy

and elevated ROS generation. From this study, the authors have concluded that the induction of HO-1, an antioxidant enzyme is an adaptation to combat oxidative stress in the myocardium of the diabetic patient, suggesting the dual role of ROS and antioxidant enzyme (HO-1) in the pathogenesis of mitochondrial cardiomyopathy in diabetes [118]. However, the mechanisms of ROS generation (sources and enzymes) and antioxidant responses are not thoroughly known in diabetic mitochondrial cardiomyopathy. The imbalance between the overwhelming production of ROS leading to oxidative stress and the antioxidant defense systems is critical in the pathogenesis of CVDs and diabetic cardiomyopathy, wherein the stress-mediated signal transduction cascades turn on the ROS generation contributing to the disease state [119]. Antioxidants appear to play a protective role against the ROS-mediated and oxidative stress-induced diabetic cardiomyopathy.

Overexpression of the mitochondrial manganese superoxide dismutase (MnSOD) in the heart of MnSOD transgenic mice with type 1 diabetes has been shown to offer protection against diabetes-induced myocardial damage such as attenuation of mitochondrial ROS, maintenance of the normal heart morphology, preservation of myocardial contractile function, and improvement of mitochondrial abundance (mass) and respiration [120]. Overall, this study clearly underscores (i) the critical role of ROS causing damage to the diabetic heart through mitochondrial dysfunction and (ii) the mitochondrial ROS (superoxide)

scavenging enzyme (MnSOD) is cardioprotective during diabetes. A study with the alloxan-induced diabetic rat model has demonstrated the enzymatic antioxidant defenses against ROS including glucose 6-phosphate dehydrogenase (G6PDH) and catalase activities and the thiol-antioxidant defense peptide, GSH in the diabetic heart mitochondria have been suppressed [121]. Furthermore, this study revealed that (i) activity of the oxygen radical-forming enzyme xanthine oxidase is enhanced in myocardium of diabetic female rats; (ii) ROS scavenging defenses in the heart mitochondria of female diabetic rats are drastically lower than in male counterparts; (iii) female diabetic rats are more severely affected than male diabetic rats; and (iv) the myocardial mitochondria appear to contribute to diabetic cardiomyopathy (more distinct in females) through suppressed ROS-scavenging defense systems. Altogether, this study demonstrated that gender plays a critical role in ROS production and altered status of the antioxidant defenses in the myocardium of the diabetic rat model in dictating the state of diabetic cardiomyopathy [121]. Nevertheless, the exact mechanisms responsible for differences in ROS production and altered antioxidant defenses in the female diabetic rat heart as compared to the same in the male diabetic rat heart warrant further investigation to flesh out gender differences in the pathogenesis of diabetic cardiomyopathy.

There have been several studies, which have clearly shown the gender differences in incidence and type of diabetes induced cardiovascular complications. The study by Juutilainen et al., with over 2,000 study subjects concluded that the diabetes-related relative risk for major cardiovascular complications is significantly increased in diabetic female compared to men. Diabetes seems to completely abolish the female protection against major CHD and the related deaths. Several other studies have demonstrated the same findings. However, the basis for the sex difference remains inconclusive [122–124]. The incidence of LV hypertrophy was also found to be at least threefold increased in diabetic female compared to men, thus contributing to cardiovascular morbidity and mortality [125].

Catalase, an important cellular antioxidant enzyme that scavenges hydrogen peroxide, has been shown to offer protection against diabetes-induced functional abnormalities, elevated levels of ROS, and apoptosis of cardiomyocytes in the myocardium of streptozotocin-induced diabetic transgenic mice overexpressing cardiac-specific catalase [126]. In addition, catalase overexpression also lowered the diabetes-mediated alterations of phospho-Akt, Foxo3a, and Sirt2 in cardiomyocytes, further suggesting that diabetes causes enhanced production of ROS. The ROS, in turn, cause alterations in critical cell signaling and epigenome-regulating enzymes that can be attenuated by the antioxidant enzyme, catalase. From this study, the authors suggest the possible use of catalase for the therapy of diabetic cardiomyopathy [126].

As studies have revealed convincing evidence for the role of ROS and ROS-mediated oxidative stress in the pathogenesis of cardiomyopathy, the exact nature, precise site/source, and regulation of ROS generation in diabetic myocardium should be elucidated to develop proper therapeutic interventions for diabetic cardiomyopathy. In this regard, several oxygen-activating and ROS-generating enzymes including NAD[P]H oxidase (NOX), xanthine oxidase, and constituents of mitochondrial electron transport in the myocardium appear to be important. NOX4, an isoform of the seven member NOX family of oxidases [127] has been shown to be upregulated in the ventricle of the streptozotocin-induced type 1 diabetic rat model with concomitant activation of NOX activity, enhancement of ROS generation, and augmented appearance of molecular markers for hypertrophy and myofibrosis [128]. Administration of the phosphorothiolated antisense for NOX4 has caused attenuation of diabetes-induced alterations in the ventricle of rats thus offering evidence for the role of NOX4 in diabetes-induced ventricular abnormalities [128]. Overall, this study strongly demonstrates that NOX4 is a critical enzyme in the generation of ROS in the ventricle, which apparently participate in the pathogenesis of diabetic cardiomyopathy. The role of NOX in HF, whether due to myocardial infarction, inflammation, drug cardiotoxicity, or diabetes needs to be thoroughly investigated [129]. Insights into the NOX enzymology in diabetic myocardium hopefully will offer new options for the prevention and therapy of HF including diabetic cardiomyopathy where in ROS is a critical player. Strategies of targeting the mitochondrial electron transport sites of ROS production with mitochondria-specific antioxidants appear as promising therapeutic option to protect against the diabetic cardiomyopathy induced by the mitochondria-generated ROS. A chief controller of intracellular redox status and detoxification process is the nuclear factor, erythroid-2-related factor 2 (Nrf2), a transcription factor belonging to the Cap'n'co'l'r/basic region leucine zipper (CNC-bZIP) family of transcription factors [130, 131]. Following activation by different oxidants and drugs, Nrf2 undergoes phosphorylation, translocates to the nucleus, binds with the antioxidant response element, and induces the expression of important cytoprotective genes including cellular antioxidant and detoxification proteins/enzymes. Nrf2 also induces activation of critical signaling cascades involved in protection against oxidant-mediated damage, immune dysregulation, inflammation, cancer, and apoptotic death [130]. Nrf2 has been identified as a chief controller or master regulator of cytoprotective mechanisms against oxidant injury, redox dysregulation, and toxicant stress, the

potential therapeutic actions in the treatment of several diseases including diabetic cardiomyopathy have been suggested [130, 131]. Coenzyme Q_{10}, a lipophilic cofactor of the mammalian mitochondrial electron transport chain, is not only crucial for mitochondrial energy production (5′-adenosine triphosphate, ATP) but also has emerged as an effective antioxidant [132]. As coenzyme Q_{10} has been recognized as a crucial player in the ATP generation in the heart, its protective actions against CVDs have been emphasized and investigated [133]. In the db/db diabetic mouse model, coenzyme Q_{10} has been observed to offer cardioprotection against the diabetes-induced hypertrophy of cardiomyocytes, ROS formation, lipoperoxidative oxidative stress, cardiac hypertrophy and remodeling and diastolic dysfunction, suggesting that coenzyme Q_{10} may be useful in the treatment of diabetic cardiomyopathy [134]. In the streptozotocin-induced type 1 diabetic mouse model, coenzyme Q_{10} administration has been shown to offer protection against diabetic cardiomyopathy mainly through attenuation of the NOX-generated ROS production in the left ventricle [134]. However, the temporal events of activation, regulation of activity, and extent of participation of different ROS-generating devices (NOX, xanthine oxidase, and mitochondrial electron transport chain) in cardiac tissue of the animal models or human subjects with diabetic cardiomyopathy need to be firmly established for effective therapeutic targeting of the source of ROS generation with pharmacological agents. Although redox signaling in the cardiovascular system has been highlighted as a critical platform for thorough investigation to unravel the intricate cascades involving redox sensor proteins and oxidative stress-mediated post-translational modifications responsible for the diabetic CVDs [135], the shortage of cardiac tissue samples (biopsies) from patients with diabetic cardiomyopathy to conduct translational studies has been a serious limitation.

Mitochondrial dysfunction in diabetic cardiomyopathy

The mitochondrion is the powerhouse of the eukaryotic cell through its use of oxidative phosphorylation to generate the cellular energy currency, ATP. This energy is essential for constant contraction of the myocardium. In addition to ATP generation, mitochondria of many tissues including the heart play crucial roles in several important physiological and pathophysiological functions such as maintenance of intracellular calcium (Ca^{2+}) levels, regulation of apoptosis, mitoptosis, formation of ROS, modulation of cellular oxidative stress, thermoregulation, autophagy, modulation of cellular signaling events, operation of the mitochondrial potassium (K^{+})-ATP channels, and engagement in the mitochondrial permeability transition pore to name a few [136–143]. Mitochondriopathy is characterized by the alterations or dysfunctions of mitochondria, which include alterations in the efficiency of respiration, energy production (ATP generation), production of ROS, antioxidant defense mechanisms, morphology and size, and mitochondrial DNA (with mutations). Hence, cardiac mitochondria have emerged as potential targets for therapeutic intervention(s) of myocardial diseases with hopes of conveying cardioprotection [144]. Diabetic cardiomyopathy appears to be no exception to the involvement of mitochondria in pathogenesis of disease.

With the use of animal models of diabetes, earlier studies have demonstrated the mitochondrial alterations in the myocardium under experimental diabetic conditions. In the isolated mitochondria from the left ventricle of streptozotocin-induced diabetic rats, state 3 respiration, oxidative phosphorylation, calcium uptake, and activity of Mg^{2+}-ATPase have been shown to be lower as compared to the same in control animals [145]. Similarly, a study performed on a diabetic male subject has revealed a tRNA(Leu) [UUR] mutation in the mitochondria with alterations in the electrocardiogram and hypertrophy of the left ventricle [146]. This case study has shown elevated number of mitochondria in the myocardial biopsies that has been correlated with the cardiac hypertrophy, cardiomyopathy, and LV systolic dysfunction. The authors have accentuated that the development of diabetic cardiomyopathy is a definite outcome of mitochondrial diabetes [146]. This study underscores that mitochondrial diabetes is a genetic predisposition to diabetic cardiomyopathy.

The critical role of cardiac mitochondrial dysfunction in the pathogenesis of cardiac diseases, including ischemia–reperfusion damage and diabetic myocardial defects has been highlighted [147]. Although several crucial factors, such as alterations in lipid metabolism, insulin resistance, and altered adipokine secretion have been recognized to play significant roles in diabetic cardiomyopathy (LV dysfunction), evidence is mounting for the role of cardiac mitochondrial abnormalities/dysfunction in the pathogenesis of diabetic cardiomyopathy [148]. Alterations or imbalances in the cardiac mitochondrial bioenergetics (energy metabolism) have been shown to be critical factors in the pathogenesis of diabetic cardiomyopathy [149, 150]. Hyperglycemia (high blood sugar) during diabetes has been recognized as a key factor in the diabetes-induced myocardial defects including diabetic cardiomyopathy through structural abnormalities in the myocardium (cardiac hypertrophy, fibrosis, myofibril defects, and cardiomyocyte aberrations) and also the myocardial mitochondrial defects such as the mitochondrial swelling and fewer number of mitochondria [151]. Oxidative stress has been noticed to be associated with the diabetes-induced myocardial structural alterations.

Mitochondrial metabolic alterations and dysfunctions of bioenergetics are being recognized as important factors in

the pathogenesis of diabetic cardiomyopathy. Elevated free fatty acid (FFA) levels (lipotoxicity) that arise under uncontrolled diabetic conditions causes substrate switch in the cardiomyocytes at the cost of glucose since the FFAs are solely taken up by the cardiomyocytes and utilized for ATP (energy) generation. The direct association between substrate switch from glucose utilization to predominant FFA utilization for energy production in the cardiomyocytes under the uncontrolled hyperglycemia and the pathogenesis of cardiomyopathy in animal (rodent) models of experimental diabetes has been recognized [152]. Additionally, the substrate switch from glucose to FFAs has been established in the myocardial mitochondria of human diabetic subjects [153]. Mitochondria isolated from the atrium of type 2 diabetic patients have shown preference for FFA utilization as the substrate for respiration, elevated levels of ROS production (H_2O_2), loss of GSH, altered redox status, and enhanced oxidative stress. This study has clearly established the substrate switch from glucose to FFAs in mitochondrial respiration and its association with oxidative stress and mitochondrial dysfunction in the myocardium of human type 2 diabetics, which may contribute to the pathogenesis of diabetic HF among humans. Furthermore, these findings suggest that the myocardial mitochondria are critical players in the pathogenesis of diabetic cardiomyopathy (hypertrophy and dysfunction of the ventricle) through the dysregulation of cardiac bioenergetics operated by the mitochondrial fuel substrate switch. Although this substrate switch appears as an adaptive strategy for the diabetic myocardial mitochondria towards ATP production during hyperglycemic stress, the mechanisms of elevation of FFAs (hydrolysis of triglycerides) and preferential uptake of FFAs by the myocardial cells (cardiomyocytes) should be thoroughly established to target the lipotoxicity-mediated diabetic cardiomyopathy at the level of mitochondrial energetics.

Altered cell signaling cascades are becoming increasingly important as critical players in the pathogenesis of diabetic cardiomyopathy. Aconitase is an important enzyme in the Krebs cycle of mitochondrial respiration and ATP synthesis. Activated protein kinase C (PKC) has been recognized as the key cell-signaling enzyme that regulates the activity of aconitase through phosphorylation in the diabetic rat myocardium. In the diabetic myocardial mitochondria, PKCβ2-mediated phosphorylation of aconitase and subsequent alterations in aconitase activity, mitochondrial functions, and bioenergetics have been observed [154]. Furthermore, this study underpins the importance of PKC-mediated myocardial mitochondrial signaling in the altered activities of aconitase in type 1 diabetic rat that could be critical in the pathogenesis of diabetic cardiomyopathy. By utilizing the insulin receptor knock-out mouse model (with cardiomyocyte deletion of insulin receptors, CIRKO), it has been shown that the loss of insulin signaling leads to uncoupling of mitochondria, which leads to elevated formation of ROS, elevated oxidative stress, decreased mitochondrial oxygen consumption with deranged respiration, alterations in pyruvate dehydrogenase, lowered ATP production, and the decline in mitochondrial bioenergetics in cardiomyocytes [155]. Thus, this study underscores the importance of cardiomyocyte insulin signaling and associated mitochondrial dysfunction towards understanding the insulin receptor/signaling-mediated diabetic cardiomyopathy. In the obese db/db mouse model with type 2 diabetes, the role of nuclear factor-κB (NF-κB) in diabetes-induced myocardial dysfunction through mitochondrial alterations has been shown [156]. Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB, has been shown to protect against the diabetes-mediated oxidative stress and mitochondrial alterations and to maintain normal ATP generation and heart function. Altogether, this study reveals the connection between NF-κB-mediated cell signaling and mitochondrial dysfunction in the diabetic myocardium that could be important in understanding the transcription factor-mediated pathogenesis of diabetic cardiomyopathy. In the streptozotocin-induced and type 2 db/db diabetic mouse models, it has been shown that the expression of p53 and synthesis of cytochrome c oxidase 2 (SCO2) have been enhanced, leading to an increase in cytochrome c oxidase (complex IV) activity, elevated oxygen consumption, and enhanced formation of ROS in cardiac mitochondria, all of which are associated with myocardial lipid buildup and dysfunction [157]. Therefore, p53 signaling in regulation of mitochondrial respiration in the diabetic heart appears to be important in the pathogenesis of diabetic cardiomyopathy. Overall, these studies have demonstrated that certain vital myocardial cellular signaling pathways intricately associated with cardiac mitochondrial function are important players in the pathogenesis of diabetic cardiomyopathy. The agonist-mediated G protein-coupled receptors (GPCRs) and the orchestrated activation of protein kinases and other signaling enzymes packaged in caveolin-containing endosomes (signalosomes) exert their actions in mitochondria and lead to cardioprotection [158]. However, the possibility of such GPCR-activated and signalosome-mediated mitochondrial protection of diabetic cardiomyopathy warrants thorough investigation. These critical signaling cascades must be better understood before therapies can be developed. More intriguing the nuclear microRNA (miRNA) (specifically miR-181c) has been shown to undergo translocation into the mitochondria of the cardiomyocytes leading to the regulation of mitochondrial expression of cytochrome *c* oxidase subunit 1 (mt-COX1) at the translational level [159]. Thus, miRNAs appear effective molecular regulators of mitochondrial

genes that could offer therapy and/or protection for diabetic cardiomyopathy.

The heart has two specific types of mitochondria, the subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM), each one with markedly distinct structure, function, and tissue location [160]. The SSM reside below the plasma membrane, whereas the IFM are localized amongst the myofibrils. Taking advantage of the distinct characteristics of the two types of myocardial mitochondria, studies have been conducted to establish the role of mitochondria in the pathogenesis of diabetic cardiomyopathy in the streptozotocin-induced diabetic mouse model [143, 160, 161]. The IFM have shown more drastic responses to the diabetic state as compared to the SSM in exhibiting decreased mitochondrial size, enhanced generation of superoxide, greater extent of oxidative stress (protein oxidation, lipid peroxidation, and nitrotyrosine formation), and reduced complex II-mediated respiration, suggesting that IFM possibly play role in diabetic cardiomyopathy [160]. The IFM have been shown to exhibit greater susceptibility to apoptosis in the heart of streptozotocin-induced diabetic mouse model as compared to the SSM in the tissue [143]. Also, expression and activity of the mitochondrial ATP-dependent potassium channel (mito-K^{+}_{ATP}) has been shown to be lowered in IFM of heart of the streptozotocin-induced diabetic mice. Overall, these studies reveal that two distinct types of myocardial mitochondria exhibit unique responses to the diabetic condition in experimental animal models, suggesting a mitochondria-driven mechanism of the pathogenesis of diabetic cardiomyopathy. However, the differential mitochondrial responses need to be established in the heart biopsy samples of human diabetic patients.

Since mitochondria play a crucial role in cell death (necrosis and apoptosis) and survival and the mitochondrial proteome exhibits changes during those stages, the utilization of mitochondrial proteome analysis in determining cardioprotection has been emphasized [162, 163]. With the use of proteomic analysis such as the two-dimensional polyacrylamide gel electrophoresis and mass spectrometry, alterations in the mitochondrial proteins of the myocardium of diabetic animal models have been studied to establish the role of mitochondria in the pathogenesis of diabetic cardiomyopathy [164, 165]. In the obesity type 2 diabetes mouse model (db/db mouse), changes in the mitochondrial proteome of the myocardium have been investigated [165]. This study revealed alterations of the mitochondrial proteins including the ATP synthase D chain, ubiquinol cytochrome *c* reductase core protein 1, and the electron transfer flavoprotein subunit α peptide along with the decrease in contractile proteins such as α-smooth muscle actin, α-cardiac actin, myosin heavy chain α, and myosin-binding protein C in the hearts of diabetic obese mice. Overall, this study suggests that alterations of mitochondrial proteins and downregulation of contractile proteins as observed in the myocardium of the obese type 2 diabetic mice appear to be involved in the diabetes-mediated aberrations of myocardial contractility [165]. Among the two different populations of myocardial mitochondria, the IFMs obtained from the type 1 diabetic heart have shown more distinct changes in the mitochondrial proteome then the SSMs, including the decline of levels of proteins of electron transport chain and fatty acid oxidation, phosphate carrier, adenine nucleotide translocator, and translocases of the mitochondrial inner membrane. Also, the study has revealed that the structural protein that is responsible for maintaining the architecture of mitochondria, mitofilin, is lowered in the IFMs of diabetic heart suggesting that diabetes causes the structural alterations of the cardiac mitochondria, which could be important in the pathogenesis of diabetic cardiomyopathy. Both the heat shock protein 70 (HSP70) and protein import in the diabetic heart, IFMs were significantly reduced. Overall, these studies have demonstrated that the changes in the cardiac mitochondrial proteome and altered protein import induced by diabetes appear to be critical in the pathogenesis of diabetic cardiomyopathy (Fig. 4). However, extensive investigations are warranted to establish the mechanisms of diabetes-associated alterations of mitochondrial proteome to target the mitochondria-driven diabetic cardiomyopathy.

While myocardial mitochondria are vital for aerobic respiration, they also are capable of generating ROS by uncoupling of electrons from the mitochondrial electron transport chain [166, 167]. Thus, mitochondria-generated ROS leads to oxidative stress, if uncontrolled by antioxidant mechanisms, leading to detrimental effects to the cardiac mitochondria and ultimately to the myocardium [167]. Therefore, ROS and oxidative stress have emerged as critical players in the pathogenesis of diabetic cardiomyopathy, wherein mitochondria have been identified as the major site of generation of ROS and targets of oxidative stress (Fig. 4). Although mitochondria are the generators of ROS, they are more susceptible to attack by ROS and exhibit dysfunction through oxidative stress [106]. Mitochondrial inner membrane contains cardiolipin, a distinctive phospholipid rich in PUFA that is susceptible to oxidative attack leading to membrane lipid peroxidation [168, 169]. Peroxidation of cardiolipin causes structural alteration and dysfunction of the cardiac mitochondria including alterations in membrane fluidity, activities of electron transport chain, and transport of ions, which ultimately lead to loss of oxidative phosphorylation ability of mitochondria and apoptotic cell death [168]. The involvement of cardiolipin peroxidation and associated elevated Ca^{2+} levels have been recognized as

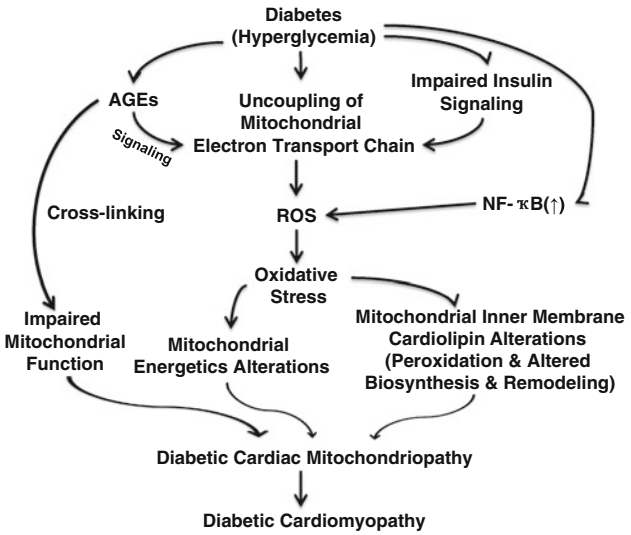

Fig. 4 Reactive oxygen species (ROS) and mitochondrial dysfunction in pathogenesis of diabetic cardiomyopathy. Hyperglycemic conditions during diabetes are known to cause uncoupling of the cardiomyocyte mitochondrial chain leading to the generation of ROS and actions of associated oxidative stress. Glucose oxidation is also known to lead to the formation of advanced glycation end products (AGEs), which form crosslinks with proteins, enzymes, and other critical macromolecules leading to the dysregulation of signaling cascades. ROS, oxidative stress, and AGEs are also known to cause alterations of the structure and function of the cardiac mitochondria leading to the overall dysfunction of the mitochondrial energetics. Cardiolipin, a unique phospholipid of the inner membrane of the mitochondria of the cardiomyocyte is vulnerable to the oxidative attack of ROS leading to the oxidative degradation of cardiolipin and dysfunction of the mitochondrial electron transport chain and oxidative phosphorylation. Thus, cardiac mitochondrial dysfunction (mitochondriopathy) appears to play a crucial role in the pathogenesis of diabetic cardiomyopathy

causative factors in the mitochondrial permeability transition and subsequent mitochondrial dysfunction, which are critical in several diseases including diabetes and CVDs [168, 170, 171]. In the heart of streptozotocin-induced diabetic animal model, drastic changes in the fatty acid molecular species and hydrolysis of cardiolipin have been observed, suggesting that the remodeling of cardiolipin could aggravate functional aberrations of mitochondria during diabetic cardiomyopathy [172]. By utilizing shotgun lipidomics in diabetic mouse heart, significant decreases in the mitochondrial (i) cardiolipin content, (ii) phosphatidylglycerol (precursor of cardiolipin) levels, and (iii) glycerol 3-phosphate (penultimate metabolite of phosphatidylglycerol biosynthesis) levels have been revealed, suggesting that cardiolipin synthesis decreases in the diabetic myocardium leading to mitochondrial dysfunction and cardiomyopathy [173]. Alterations in cardiolipin-synthesizing/remodeling enzymes in heart during the pathogenesis of HF have been shown [174]. Cardiolipin synthase has been identified as a new molecular target to mitigate diabetic cardiac mitochondrial dysfunction [175]. Hence, the regulation of cardiolipin metabolism (synthesis, turnover, and remodeling) during the pathogenesis of diabetic cardiomyopathy at temporal and spatial (SSM and IFM) levels should be thoroughly studied to pinpoint the alterations/dysfunctions of the diabetic heart. This is likely to offer insights into specific cardiac mitochondrial targets focusing on cardiolipin towards effective therapeutic treatments of diabetic cardiomyopathy in humans.

Although evidence is mounting for the role/contribution of mitochondrial dysfunction in the pathogenesis of several debilitating diseases including the myocardial pathologies, no appropriate or effective treatments are currently available to correct or treat those mitochondriopathies. Most of the existing or available therapeutic options hinge upon empirical data and experience of the clinician so there is a pressing need for controlled clinical trials that lead to evidence-based treatments such as the mitochondria-targeted antioxidants and co-enzyeme Q10, for mitochondrial pathologies, including diabetic cardiomyopathy [176].

Conclusions

The mechanisms of pathogenesis of diabetic cardiomyopathy clearly appear to be complex and involve dysfunction and damage of LV tissue, cardiac hypertrophy, myocardial fibrosis, alterations of cardiomyocytes, microangiopathy, changes in coronary vessels, and vascular EC derangements. It is becoming clear that the mechanism(s) of diabetic neuropathy are orchestrated by the involvement of ROS, oxidative stress, and mitochondrial dysfunction. More detail is needed to firmly establish the mitochondrial and/or ROS mechanism(s) behind diabetic cardiomyopathy by identifying the most critical molecular players involved at both spatial and temporal levels of pathophysiology as targets for specific and effective pharmacological/therapeutic interventions.

Acknowledgments This study was supported by National Institute of Health Grants HL-56803 and HL-69910 to NM. We would like to acknowledge Shereen Cynthia D'Cruz, Ph.D and Joshua Goldman for their assistance during the preparation of the manuscript.

References

1. Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14
2. Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G (2010) Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:293–301

3. Giacomelli F, Wiener J (1979) Primary myocardial disease in the diabetic mouse. An ultrastructural study. Lab Invest 40:460–473
4. Roda L, Patessio A, Neri V, Tisi C, Ferrari A, Ricci A (1980) Diabetic cardiomyopathy in preclinical phase. Polycardiographic and echocardiographic study (author's transl). G Ital Cardiol 10:1299–1307
5. Scott RC (1984) Cardiomyopathy in diabetic patients. West J Med 140:610–612
6. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A (1972) New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am J Cardiol 30:595–602
7. Liu JE, Palmieri V, Roman MJ, Bella JN, Fabsitz R, Howard BV, Welty TK, Lee ET, Devereux RB (2001) The impact of diabetes on left ventricular filling pattern in normotensive and hypertensive adults: the Strong Heart Study. J Am Coll Cardiol 37:1943–1949
8. Kannel WB, Hjortland M, Castelli WP (1974) Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 34:29–34
9. Aronow WS, Ahn C (1999) Incidence of heart failure in 2,737 older persons with and without diabetes mellitus. Chest 115: 867–868
10. Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB (2004) The incidence of congestive heart failure in type 2 diabetes: an update. Diabetes Care 27:1879–1884
11. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321:405–412
12. Garcia MJ, McNamara PM, Gordon T, Kannel WB (1974) Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. Diabetes 23:105–111
13. Gaede P, Vedel P, Parving HH, Pedersen O (1999) Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. Lancet 353:617–622
14. Poornima IG, Parikh P, Shannon RP (2006) Diabetic cardiomyopathy: the search for a unifying hypothesis. Circ Res 98:596–605
15. Raev DC (1994) Left ventricular function and specific diabetic complications in other target organs in young insulin-dependent diabetics: an echocardiographic study. Heart Vessels 9:121–128
16. Schannwell CM, Schoebel FC, Heggen S, Marx R, Perings C, Leschke M, Strauer BE (1999) Early decrease in diastolic function in young type I diabetic patients as an initial manifestation of diabetic cardiomyopathy. Z Kardiol 88:338–346
17. Maya L, Villarreal FJ (2010) Diagnostic approaches for diabetic cardiomyopathy and myocardial fibrosis. J Mol Cell Cardiol 48:524–529
18. Khouri SJ, Maly GT, Suh DD, Walsh TE (2004) A practical approach to the echocardiographic evaluation of diastolic function. J Am Soc Echocardiogr 17:290–297
19. Di Bonito P, Moio N, Cavuto L, Covino G, Murena E, Scilla C, Turco S, Capaldo B, Sibilio G (2005) Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging. Diabet Med 22:1720–1725
20. Boyer JK, Thanigaraj S, Schechtman KB, Perez JE (2004) Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. Am J Cardiol 93:870–875
21. Saraiva RM, Duarte DM, Duarte MP, Martins AF, Poltronieri AV, Ferreira ME, Silva MC, Hohleuwerger R, Ellis A, Rachid MB, Monteiro CF, Kaiser SE (2005) Tissue Doppler imaging identifies asymptomatic normotensive diabetics with diastolic dysfunction and reduced exercise tolerance. Echocardiography 22:561–570
22. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG (2001) Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 24:5–10
23. Raev DC (1994) Which left ventricular function is impaired earlier in the evolution of diabetic cardiomyopathy? An echocardiographic study of young type I diabetic patients. Diabetes Care 17:633–639
24. Von Bibra H, Thrainsdottir IS, Hansen A, Dounis V, Malmberg K, Ryden L (2005) Tissue Doppler imaging for the detection and quantitation of myocardial dysfunction in patients with type 2 diabetes mellitus. Diab Vasc Dis Res 2:24–30
25. Zabalgoitia M, Ismaeil MF, Anderson L, Maklady FA (2001) Prevalence of diastolic dysfunction in normotensive, asymptomatic patients with well-controlled type 2 diabetes mellitus. Am J Cardiol 87:320–323
26. Henry RM, Paulus WJ, Kamp O, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD (2008) Deteriorating glucose tolerance status is associated with left ventricular dysfunction—the Hoorn Study. Neth J Med 66:110–117
27. Kane GC, Karon BL, Mahoney DW, Redfield MM, Roger VL, Burnett JC Jr, Jacobsen SJ, Rodeheffer RJ (2011) Progression of left ventricular diastolic dysfunction and risk of heart failure. JAMA 306:856–863
28. Astorri E, Fiorina P, Contini GA, Albertini D, Magnati G, Astorri A, Lanfredini M (1997) Isolated and preclinical impairment of left ventricular filling in insulin-dependent and non-insulin-dependent diabetic patients. Clin Cardiol 20:536–540
29. Robillon JF, Sadoul JL, Jullien D, Morand P, Freychet P (1994) Abnormalities suggestive of cardiomyopathy in patients with type 2 diabetes of relatively short duration. Diabete Metab 20:473–480
30. Salazar J, Rivas A, Rodriguez M, Felipe J, Garcia MD, Bone J (1994) Left ventricular function determined by Doppler echocardiography in adolescents with type I (insulin-dependent) diabetes mellitus. Acta Cardiol 49:435–439
31. Dinh W, Lankisch M, Nickl W, Gies M, Scheyer D, Kramer F, Scheffold T, Krahns T, Sause A, Futh R (2011) Metabolic syndrome with or without diabetes contributes to left ventricular diastolic dysfunction. Acta Cardiol 66:167–174
32. Dinh W, Lankisch M, Nickl W, Scheyer D, Scheffold T, Kramer F, Krahn T, Klein RM, Barroso MC, Futh R (2010) Insulin resistance and glycemic abnormalities are associated with deterioration of left ventricular diastolic function: a cross-sectional study. Cardiovasc Diabetol 9:63
33. Futh R, Dinh W, Bansemir L, Ziegler G, Bufe A, Wolfertz J, Scheffold T, Lankisch M (2009) Newly detected glucose disturbance is associated with a high prevalence of diastolic dysfunction: double risk for the development of heart failure? Acta Diabetol 46:335–338
34. Shimabukuro M, Higa N, Asahi T, Yamakawa K, Oshiro Y, Higa M, Masuzaki H (2011) Impaired glucose tolerance, but not impaired fasting glucose, underlies left ventricular diastolic dysfunction. Diabetes Care 34:686–690
35. Di Cori A, Di Bello V, Miccoli R, Talini E, Palagi C, Delle Donne MG, Penno G, Nardi C, Bianchi C, Mariani M, Del Prato S, Balbarini A (2007) Left ventricular function in normotensive young adults with well-controlled type 1 diabetes mellitus. Am J Cardiol 99:84–90
36. Ha JW, Oh JK, Pellikka PA, Ommen SR, Stussy VL, Bailey KR, Seward JB, Tajik AJ (2005) Diastolic stress echocardiography: a novel noninvasive diagnostic test for diastolic dysfunction using supine bicycle exercise Doppler echocardiography. J Am Soc Echocardiogr 18:63–68

37. Ernande L, Rietzschel ER, Bergerot C, De Buyzere ML, Schnell F, Groisne L, Ovize M, Croisille P, Moulin P, Gillebert TC, Derumeaux G (2010) Impaired myocardial radial function in asymptomatic patients with type 2 diabetes mellitus: a speckle-tracking imaging study. J Am Soc Echocardiogr 23:1266–1272
38. Acar G, Akcay A, Sokmen A, Ozkaya M, Guler E, Sokmen G, Kaya H, Nacar AB, Tuncer C (2009) Assessment of atrial electromechanical delay, diastolic functions, and left atrial mechanical functions in patients with type 1 diabetes mellitus. J Am Soc Echocardiogr 22:732–738
39. Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, Kitzman D (2001) Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the Cardiovascular Health Study. J Am Coll Cardiol 37:1042–1048
40. Eren M, Gorgulu S, Uslu N, Celik S, Dagdeviren B, Tezel T (2004) Relation between aortic stiffness and left ventricular diastolic function in patients with hypertension, diabetes, or both. Heart 90:37–43
41. Mitchell GF, Moyé LA, Braunwald E, Rouleau J-L, Bernstein V, Geltman EM, Flaker GC, Pfeffer MA (1997) Sphygmomanometrically determined pulse pressure is a powerful independent predictor of recurrent events after myocardial infarction in patients with impaired left ventricular function. Circulation 96:4254–4260
42. Doering CW, Jalil JE, Janicki JS, Pick R, Aghili S, Abrahams C, Weber K (1988) Collagen network remodelling and diastolic stiffness of the rat left ventricle with pressure overload hypertrophy. Cardiovasc Res 22:686–695
43. Taegtmeyer H, McNulty P, Young ME (2002) Adaptation and maladaptation of the heart in diabetes: part I General concepts. Circulation 105:1727–1733
44. Mahgoub MA, Abd-Elfattah AS (1998) Diabetes mellitus and cardiac function. Mol Cell Biochem 180:59–64
45. Van den Bergh A, Flameng W, Herijgers P (2006) Type II diabetic mice exhibit contractile dysfunction but maintain cardiac output by favourable loading conditions. Eur J Heart Fail 8:777–783
46. Radovits T, Korkmaz S, Loganathan S, Barnucz E, Bomicke T, Arif R, Karck M, Szabo G (2009) Comparative investigation of the left ventricular pressure–volume relationship in rat models of type 1 and type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 297:H125–H133
47. Boudina S, Abel ED (2007) Diabetic cardiomyopathy revisited. Circulation 115:3213–3223
48. Fischer VW, Barner HB, LaRose LS (1982) Quadriceps and myocardial capillary basal laminae. Their comparison in diabetic patients. Arch Pathol Lab Med 106:336–341
49. Fischer VW, Barner HB, Larose LS (1984) Pathomorphologic aspects of muscular tissue in diabetes mellitus. Hum Pathol 15:1127–1136
50. Sutherland CG, Fisher BM, Frier BM, Dargie HJ, More IA, Lindop GB (1989) Endomyocardial biopsy pathology in insulin-dependent diabetic patients with abnormal ventricular function. Histopathology 14:593–602
51. Kawaguchi M, Techigawara M, Ishihata T, Asakura T, Saito F, Maehara K, Maruyama Y (1997) A comparison of ultrastructural changes on endomyocardial biopsy specimens obtained from patients with diabetes mellitus with and without hypertension. Heart Vessels 12:267–274
52. Thompson EW (1988) Structural manifestations of diabetic cardiomyopathy in the rat and its reversal by insulin treatment. Am J Anat 182:270–282
53. Brownlee M, Cerami A, Vlassara H (1988) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 318:1315–1321
54. Candido R, Forbes JM, Thomas MC, Thallas V, Dean RG, Burns WC, Tikellis C, Ritchie RH, Twigg SM, Cooper ME, Burrell LM (2003) A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ Res 92:785–792
55. Norton GR, Candy G, Woodiwiss AJ (1996) Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. Circulation 93:1905–1912
56. Sharma S, Adrogue JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H (2004) Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J 18:1692–1700
57. Rohr S (2004) Role of gap junctions in the propagation of the cardiac action potential. Cardiovasc Res 62:309–322
58. Berridge MJ (1997) Elementary and global aspects of calcium signalling. J Exp Biol 200:315–319
59. Bers DM (2002) Cardiac excitation–contraction coupling. Nature 415:198–205
60. Choi KM, Zhong Y, Hoit BD, Grupp IL, Hahn H, Dilly KW, Guatimosim S, Lederer WJ, Matlib MA (2002) Defective intracellular Ca(2+) signaling contributes to cardiomyopathy in Type 1 diabetic rats. Am J Physiol Heart Circ Physiol 283:H1398–H1408
61. op den Buijs J, Miklos Z, van Riel NA, Prestia CM, Szenczi O, Toth A, Van der Vusse GJ, Szabo C, Ligeti L, Ivanics T (2005) beta-Adrenergic activation reveals impaired cardiac calcium handling at early stage of diabetes. Life Sci 76:1083–1098
62. Ren J, Davidoff AJ (1997) Diabetes rapidly induces contractile dysfunctions in isolated ventricular myocytes. Am J Physiol 272:H148–H158
63. Ganguly PK, Pierce GN, Dhalla KS, Dhalla NS (1983) Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am J Physiol 244:E528–E535
64. Penpargkul S, Fein F, Sonnenblick EH, Scheuer J (1981) Depressed cardiac sarcoplasmic reticular function from diabetic rats. J Mol Cell Cardiol 13:303–309
65. Zhao XY, Hu SJ, Li J, Mou Y, Chen BP, Xia Q (2006) Decreased cardiac sarcoplasmic reticulum Ca^{2+}-ATPase activity contributes to cardiac dysfunction in streptozotocin-induced diabetic rats. J Physiol Biochem 62:1–8
66. Teshima Y, Takahashi N, Saikawa T, Hara M, Yasunaga S, Hidaka S, Sakata T (2000) Diminished expression of sarcoplasmic reticulum Ca(2+)-ATPase and ryanodine sensitive Ca(2+)Channel mRNA in streptozotocin-induced diabetic rat heart. J Mol Cell Cardiol 32:655–664
67. Zhong Y, Ahmed S, Grupp IL, Matlib MA (2001) Altered SR protein expression associated with contractile dysfunction in diabetic rat hearts. Am J Physiol Heart Circ Physiol 281:H1137–H1147
68. Bidasee KR, Dincer UD, Besch HR Jr (2001) Ryanodine receptor dysfunction in hearts of streptozotocin-induced diabetic rats. Mol Pharmacol 60:1356–1364
69. Trost SU, Belke DD, Bluhm WF, Meyer M, Swanson E, Dillmann WH (2002) Overexpression of the sarcoplasmic reticulum Ca(2+)-ATPase improves myocardial contractility in diabetic cardiomyopathy. Diabetes 51:1166–1171
70. Belke DD, Swanson EA, Dillmann WH (2004) Decreased sarcoplasmic reticulum activity and contractility in diabetic db/db mouse heart. Diabetes 53:3201–3208
71. Yaras N, Ugur M, Ozdemir S, Gurdal H, Purali N, Lacampagne A, Vassort G, Turan B (2005) Effects of diabetes on ryanodine receptor Ca release channel (RyR2) and Ca^{2+} homeostasis in rat heart. Diabetes 54:3082–3088
72. Vita JA, Keaney JF Jr (2002) Endothelial function: a barometer for cardiovascular risk? Circulation 106:640–642

73. Drexler H (1998) Factors involved in the maintenance of endothelial function. Am J Cardiol 82:3S–4S
74. Meigs JB, Hu FB, Rifai N, Manson JE (2004) Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 291:1978–1986
75. Schalkwijk C, Stehouwer C (2005) Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci 109:143–159
76. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
77. Forstermann U, Munzel T (2006) Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113:1708–1714
78. Li H, Förstermann U (2000) Nitric oxide in the pathogenesis of vascular disease. J Pathol 190:244–254
79. Chaudhuri G, Cuevas J, Buga GM, Ignarro LJ (1993) NO is more important than PGI2 in maintaining low vascular tone in feto-placental vessels. Am J Physiol Heart Circ Physiol 265:H2036–H2043
80. Hamed S, Brenner B, Aharon A, Daoud D, Roguin A (2009) Nitric oxide and superoxide dismutase modulate endothelial progenitor cell function in type 2 diabetes mellitus. Cardiovasc Diabetol 8:56
81. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM (2002) Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 51:1131–1137
82. Hirata K, Kuroda R, Sakoda T, Katayama M, Inoue N, Suematsu M, Kawashima S, Yokoyama M (1995) Inhibition of endothelial nitric oxide synthase activity by protein kinase C. Hypertension 25:180–185
83. Frank PG, Lisanti MP (2008) ICAM-1: role in inflammation and in the regulation of vascular permeability. Am J Physiol Heart Circ Physiol 295:H926–H927
84. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P (1986) Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 315:1046–1051
85. Adameova A, Dhalla NS (2013) Role of microangiopathy in diabetic cardiomyopathy. Heart Fail Rev. doi:10.1007/s10741-013-9378-7
86. Mortuza R, Chakrabarti S (2013) Glucose-induced cell signaling in the pathogenesis of diabetic cardiomyopathy. Heart Fail Rev. doi:10.1007/s10741-013-9381-z
87. Sheikh AQ, Hurley JR, Huang W, Taghian T, Kogan A, Cho H, Wang Y, Narmoneva DA (2012) Diabetes alters intracellular calcium transients in cardiac endothelial cells. PLoS ONE 7:e36840
88. Cheng Y, Guo S, Liu G, Feng Y, Yan B, Yu J, Feng K, Li Z (2012) Transplantation of bone marrow-derived endothelial progenitor cells attenuates myocardial interstitial fibrosis and cardiac dysfunction in streptozotocin-induced diabetic rats. Int J Mol Med 30:870–876
89. Duvall WL (2005) Endothelial dysfunction and antioxidants. Mt Sinai J Med 72:71–80
90. Monti LD, Casiraghi MC, Setola E, Galluccio E, Pagani MA, Quaglia L, Bosi E, Piatti P (2013) L-Arginine enriched biscuits improve endothelial function and glucose metabolism: a pilot study in healthy subjects and a cross-over study in subjects with impaired glucose tolerance and metabolic syndrome. Metabolism 62:255–264
91. Shechter M, Sharir M, Labrador MJ, Forrester J, Silver B, Bairey Merz CN (2000) Oral magnesium therapy improves endothelial function in patients with coronary artery disease. Circulation 102:2353–2358
92. Kawano H, Yasue H, Kitagawa A, Hirai N, Yoshida T, Soejima H, Miyamoto S, Nakano M, Ogawa H (2003) Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. J Clin Endocrinol Metab 88:3190–3195
93. Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim J-a, Quon MJ, Montagnani M (2007) EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 292:E1378–E1387
94. Hirata Y, Nagata D, Suzuki E, Nishimatsu H, Suzuki J, Nagai R (2010) Diagnosis and treatment of endothelial dysfunction in cardiovascular disease. Int Heart J 51:1–6
95. Kulkarni AC, Kuppusamy P, Parinandi N (2007) Oxygen, the lead actor in the pathophysiologic drama: enactment of the trinity of normoxia, hypoxia, and hyperoxia in disease and therapy. Antioxid Redox Signal 9:1717–1730
96. Freinbichler W, Colivicchi MA, Stefanini C, Bianchi L, Ballini C, Misini B, Weinberger P, Linert W, Vareslija D, Tipton KF, Della Corte L (2011) Highly reactive oxygen species: detection, formation, and possible functions. Cell Mol Life Sci 68:2067–2079
97. Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D (2013) Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr Vasc Pharmacol. PMID: 23628007
98. Sultana R, Perluigi M, Allan Butterfield D (2013) Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radic Biol Med 62:157–169
99. Xu Y, Gu Y, Qian SY (2012) An advanced electron spin resonance (ESR) spin-trapping and LC/(ESR)/MS technique for the study of lipid peroxidation. Int J Mol Sci 13:14648–14666
100. Bocci V, Valacchi G (2013) Free radicals and antioxidants: how to reestablish redox homeostasis in chronic diseases? Curr Med Chem
101. Guerra-Araiza C, Alvarez-Mejia AL, Sanchez-Torres S, Farfan-Garcia E, Mondragon-Lozano R, Pinto-Almazan R, Salgado-Ceballos H (2013) Effect of natural exogenous antioxidants on aging and on neurodegenerative diseases. Free Radic Res 47:451–462
102. Smeyne M, Smeyne RJ (2013) Glutathione metabolism and Parkinson disease. Free Radic Biol Med 62:13–25
103. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19
104. Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91 Spec No:179–194
105. Giergiel M, Lopucki M, Stachowicz N, Kankofer M (2012) The influence of age and gender on antioxidant enzyme activities in humans and laboratory animals. Aging Clin Exp Res 24:561–569
106. Wang J, Song Y, Wang Q, Kralik PM, Epstein PN (2006) Causes and characteristics of diabetic cardiomyopathy. Rev Diabet Stud 3:108–117
107. Falcao-Pires I, Leite-Moreira AF (2012) Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. Heart Fail Rev 17:325–344
108. Khavandi K, Khavandi A, Asghar O, Greenstein A, Withers S, Heagerty AM, Malik RA (2009) Diabetic cardiomyopathy—a distinct disease? Best Pract Res Clin Endocrinol Metab 23:347–360
109. Ungvari Z, Gupte SA, Recchia FA, Batkai S, Pacher P (2005) Role of oxidative-nitrosative stress and downstream pathways in various forms of cardiomyopathy and heart failure. Curr Vasc Pharmacol 3:221–229

110. Haidara MA, Yassin HZ, Rateb M, Ammar H, Zorkani MA (2006) Role of oxidative stress in development of cardiovascular complications in diabetes mellitus. Curr Vasc Pharmacol 4:215–227
111. Selvaraju V, Joshi M, Suresh S, Sanchez JA, Maulik N, Maulik G (2012) Diabetes, oxidative stress, molecular mechanism, and cardiovascular disease—an overview. Toxicol Mech Methods 22:330–335
112. Khullar M, Al-Shudiefat AA, Ludke A, Binepal G, Singal PK (2010) Oxidative stress: a key contributor to diabetic cardiomyopathy. Can J Physiol Pharmacol 88:233–240
113. Ansley DM, Wang B (2013) Oxidative stress and myocardial injury in the diabetic heart. J Pathol 229:232–241
114. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107:1058–1070
115. Mellor KM, Ritchie RH, Delbridge LM (2010) Reactive oxygen species and insulin-resistant cardiomyopathy. Clin Exp Pharmacol Physiol 37:222–228
116. Dirkx E, Schwenk RW, Glatz JF, Luiken JJ, van Eys GJ (2011) High fat diet induced diabetic cardiomyopathy. Prostaglandins Leukot Essent Fatty Acids 85:219–225
117. Cai L, Kang YJ (2001) Oxidative stress and diabetic cardiomyopathy: a brief review. Cardiovasc Toxicol 1:181–193
118. Ishikawa K, Kimura S, Kobayashi A, Sato T, Matsumoto H, Ujiie Y, Nakazato K, Mitsugi M, Maruyama Y (2005) Increased reactive oxygen species and anti-oxidative response in mitochondrial cardiomyopathy. Circ J 69:617–620
119. Wold LE, Ceylan-Isik AF, Ren J (2005) Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. Acta Pharmacol Sin 26:908–917
120. Shen X, Zheng S, Metreveli NS, Epstein PN (2006) Protection of cardiac mitochondria by overexpression of MnSOD reduces diabetic cardiomyopathy. Diabetes 55:798–805
121. Akhileshwar V, Patel SP, Katyare SS (2007) Diabetic cardiomyopathy and reactive oxygen species (ROS) related parameters in male and female rats: a comparative study. Indian J Clin Biochem 22:84–90
122. Almdal T, Scharling H, Jensen JS, Vestergaard H (2004) The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. Arch Intern Med 164:1422–1426
123. Barrett-Connor E, Giardina EG, Gitt AK, Gudat U, Steinberg HO, Tschoepe D (2004) Women and heart disease: the role of diabetes and hyperglycemia. Arch Intern Med 164:934–942
124. Juutilainen A, Kortelainen S, Lehto S, Ronnemaa T, Pyorala K, Laakso M (2004) Gender difference in the impact of type 2 diabetes on coronary heart disease risk. Diabetes Care 27:2898–2904
125. Bruno G, Giunti S, Bargero G, Ferrero S, Pagano G, Perin PC (2004) Sex-differences in prevalence of electrocardiographic left ventricular hypertrophy in Type 2 diabetes: the Casale Monferrato Study. Diabet Med 21:823–828
126. Turdi S, Li Q, Lopez FL, Ren J (2007) Catalase alleviates cardiomyocyte dysfunction in diabetes: role of Akt, Forkhead transcriptional factor and silent information regulator 2. Life Sci 81:895–905
127. Montezano AC, Touyz RM (2012) Reactive oxygen species and endothelial function—role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases. Basic Clin Pharmacol Toxicol 110:87–94
128. Maalouf RM, Eid AA, Gorin YC, Block K, Escobar GP, Bailey S, Abboud HE (2012) Nox4-derived reactive oxygen species mediate cardiomyocyte injury in early type 1 diabetes. Am J Physiol Cell Physiol 302:C597–C604
129. Octavia Y, Brunner-La Rocca HP, Moens AL (2012) NADPH oxidase-dependent oxidative stress in the failing heart: from pathogenic roles to therapeutic approach. Free Radic Biol Med 52:291–297
130. Cho HY, Reddy SP, Kleeberger SR (2006) Nrf2 defends the lung from oxidative stress. Antioxid Redox Signal 8:76–87
131. Li B, Liu S, Miao L, Cai L (2012) Prevention of diabetic complications by activation of Nrf2: diabetic cardiomyopathy and nephropathy. Exp Diabetes Res 2012:216512
132. Bhagavan HN, Chopra RK (2007) Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations. Mitochondrion 7(Suppl):S78–S88
133. Littarru GP, Tiano L, Belardinelli R, Watts GF (2011) Coenzyme Q(10), endothelial function, and cardiovascular disease. BioFactors 37:366–373
134. Huynh K, Kiriazis H, Du XJ, Love JE, Gray SP, Jandeleit-Dahm KA, McMullen JR, Ritchie RH (2013) Targeting the upregulation of reactive oxygen species subsequent to hyperglycemia prevents type 1 diabetic cardiomyopathy in mice. Free Radic Biol Med 60:307–317
135. Turan B (2010) Role of antioxidants in redox regulation of diabetic cardiovascular complications. Curr Pharm Biotechnol 11:819–836
136. Apostolova N, Blas-Garcia A, Esplugues JV (2011) Mitochondria sentencing about cellular life and death: a matter of oxidative stress. Curr Pharm Des 17:4047–4060
137. Higgins GC, Beart PM, Shin YS, Chen MJ, Cheung NS, Nagley P (2010) Oxidative stress: emerging mitochondrial and cellular themes and variations in neuronal injury. J Alzheimers Dis 20(Suppl 2):S453–S473
138. Holmuhamedov EL, Jovanovic S, Dzeja PP, Jovanovic A, Terzic A (1998) Mitochondrial ATP-sensitive K^+ channels modulate cardiac mitochondrial function. Am J Physiol 275:H1567–H1576
139. Kowaltowski AJ (2000) Alternative mitochondrial functions in cell physiopathology: beyond ATP production. Braz J Med Biol Res 33:241–250
140. Mammucari C, Rizzuto R (2010) Signaling pathways in mitochondrial dysfunction and aging. Mech Ageing Dev 131: 536–543
141. Scherz-Shouval R, Elazar Z (2007) ROS, mitochondria and the regulation of autophagy. Trends Cell Biol 17:422–427
142. Sordahl LA (1979) Role of mitochondria in heart cell function. Tex Rep Biol Med 39:5–18
143. Williamson CL, Dabkowski ER, Baseler WA, Croston TL, Alway SE, Hollander JM (2010) Enhanced apoptotic propensity in diabetic cardiac mitochondria: influence of subcellular spatial location. Am J Physiol Heart Circ Physiol 298:H633–H642
144. Di Lisa F, Canton M, Menabo R, Kaludercic N, Bernardi P (2007) Mitochondria and cardioprotection. Heart Fail Rev 12:249–260
145. Tanaka Y, Konno N, Kako KJ (1992) Mitochondrial dysfunction observed in situ in cardiomyocytes of rats in experimental diabetes. Cardiovasc Res 26:409–414
146. Momiyama Y, Atsumi Y, Ohsuzu F, Ui S, Morinaga S, Matsuoka K, Kimura M (1999) Rapid progression of cardiomyopathy in mitochondrial diabetes. Jpn Circ J 63:130–132
147. Sack MN (2009) Type 2 diabetes, mitochondrial biology and the heart. J Mol Cell Cardiol 46:842–849
148. Dobrin JS, Lebeche D (2010) Diabetic cardiomyopathy: signaling defects and therapeutic approaches. Expert Rev Cardiovasc Ther 8:373–391
149. Bugger H, Abel ED (2010) Mitochondria in the diabetic heart. Cardiovasc Res 88:229–240
150. Duncan JG (2011) Mitochondrial dysfunction in diabetic cardiomyopathy. Biochim Biophys Acta 1813:1351–1359

151. Adeghate E, Singh J (2013) Structural changes in the myocardium during diabetes-induced cardiomyopathy. Heart Fail Rev. doi:10.1007/s10741-013-9388-5
152. Feuvray D, Darmellah A (2008) Diabetes-related metabolic perturbations in cardiac myocyte. Diabetes Metab 34(Suppl 1): S3–S9
153. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD (2009) Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. J Am Coll Cardiol 54:1891–1898
154. Lin G, Brownsey RW, MacLeod KM (2009) Regulation of mitochondrial aconitase by phosphorylation in diabetic rat heart. Cell Mol Life Sci 66:919–932
155. Boudina S, Bugger H, Sena S, O'Neill BT, Zaha VG, Ilkun O, Wright JJ, Mazumder PK, Palfreyman E, Tidwell TJ, Theobald H, Khalimonchuk O, Wayment B, Sheng X, Rodnick KJ, Centini R, Chen D, Litwin SE, Weimer BE, Abel ED (2009) Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart. Circulation 119:1272–1283
156. Mariappan N, Elks CM, Sriramula S, Guggilam A, Liu Z, Borkhsenious O, Francis J (2010) NF-kappaB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes. Cardiovasc Res 85:473–483
157. Nakamura H, Matoba S, Iwai-Kanai E, Kimata M, Hoshino A, Nakaoka M, Katamura M, Okawa Y, Ariyoshi M, Mita Y, Ikeda K, Okigaki M, Adachi S, Tanaka H, Takamatsu T, Matsubara H (2012) p53 promotes cardiac dysfunction in diabetic mellitus caused by excessive mitochondrial respiration-mediated reactive oxygen species generation and lipid accumulation. Circ Heart Fail 5:106–115
158. Murphy E, Wong R, Steenbergen C (2008) Signalosomes: delivering cardioprotective signals from GPCRs to mitochondria. Am J Physiol Heart Circ Physiol 295:H920–H922
159. Latronico MV, Condorelli G (2012) The might of microRNA in mitochondria. Circ Res 110:1540–1542
160. Dabkowski ER, Williamson CL, Bukowski VC, Chapman RS, Leonard SS, Peer CJ, Callery PS, Hollander JM (2009) Diabetic cardiomyopathy-associated dysfunction in spatially distinct mitochondrial subpopulations. Am J Physiol Heart Circ Physiol 296:H359–H369
161. Fancher IS, Dick GM, Hollander JM (2013) Diabetes mellitus reduces the function and expression of ATP-dependent K(+) channels in cardiac mitochondria. Life Sci 92:664–668
162. Gucek M, Murphy E (2010) What can we learn about cardioprotection from the cardiac mitochondrial proteome? Cardiovasc Res 88:211–218
163. Murphy E, Steenbergen C (2011) What makes the mitochondria a killer? Can we condition them to be less destructive? Biochim Biophys Acta 1813:1302–1308
164. Baseler WA, Dabkowski ER, Williamson CL, Croston TL, Thapa D, Powell MJ, Razunguzwa TT, Hollander JM (2011) Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart: contribution of protein import dysfunction. Am J Physiol Regul Integr Comp Physiol 300:R186–R200
165. Essop MF, Chan WA, Hattingh S (2011) Proteomic analysis of mitochondrial proteins in a mouse model of type 2 diabetes. Cardiovasc J Afr 22:175–178
166. Bolisetty S, Jaimes EA (2013) Mitochondria and reactive oxygen species: physiology and pathophysiology. Int J Mol Sci 14:6306–6344
167. Gao L, Laude K, Cai H (2008) Mitochondrial pathophysiology, reactive oxygen species, and cardiovascular diseases. Vet Clin North Am Small Anim Pract 38:137–155, vi
168. Paradies G, Petrosillo G, Paradies V, Ruggiero FM (2009) Role of cardiolipin peroxidation and Ca^{2+} in mitochondrial dysfunction and disease. Cell Calcium 45:643–650
169. Parinandi NL, Weis BK, Schmid HH (1988) Assay of cardiolipin peroxidation by high-performance liquid chromatography. Chem Phys Lipids 49:215–220
170. Shi Y (2010) Emerging roles of cardiolipin remodeling in mitochondrial dysfunction associated with diabetes, obesity, and cardiovascular diseases. J Biomed Res 24:6–15
171. Wong R, Steenbergen C, Murphy E (2012) Mitochondrial permeability transition pore and calcium handling. Methods Mol Biol 810:235–242
172. Han X, Yang J, Yang K, Zhao Z, Abendschein DR, Gross RW (2007) Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. Biochemistry 46:6417–6428
173. Han X, Yang J, Cheng H, Yang K, Abendschein DR, Gross RW (2005) Shotgun lipidomics identifies cardiolipin depletion in diabetic myocardium linking altered substrate utilization with mitochondrial dysfunction. Biochemistry 44:16684–16694
174. Saini-Chohan HK, Holmes MG, Chicco AJ, Taylor WA, Moore RL, McCune SA, Hickson-Bick DL, Hatch GM, Sparagna GC (2009) Cardiolipin biosynthesis and remodeling enzymes are altered during development of heart failure. J Lipid Res 50:1600–1608
175. Kiebish MA, Yang K, Sims HF, Jenkins CM, Liu X, Mancuso DJ, Zhao Z, Guan S, Abendschein DR, Han X, Gross RW (2012) Myocardial regulation of lipidomic flux by cardiolipin synthase: setting the beat for bioenergetic efficiency. J Biol Chem 287:25086–25097
176. Goldstein A, Wolfe LA (2013) The elusive magic pill: finding effective therapies for mitochondrial disorders. Neurotherapeutics 10:320–328