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Mahmoud E. Youssef
Delta University for Science and Technology

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MOLECULAR MECHANISMS AND SIGNALING PATHWAYS OF DIABETIC CARDIOMYOPATHY

Mahmoud E. Youssef
Assistant lecturer of pharmacology and toxicology, department of pharmacology and biochemistry, Delta University for Science and Technology

Abstract

Diabetes and heart failure incidence are increasing worldwide and responsible for high morbidity and mortality rates. The clinical outcomes arising from diabetic complications such as nephropathy, neuropathy, retinopathy and cardiomyopathy are more serious. This review focuses on diabetic cardiomyopathy clinical aspects and disease prognosis. Additionally, it focuses on the different molecular and protein signalling pathways involved in diabetic cardiomyopathy including the increased mitochondrial oxidative stress, AGE and RAGE pathways, activated RAAS, Impaired AMPK, alterations in PPARs, SGLT2 abnormalities, PKC activation MAPK and JNK alterations, activation of NF-κB. targeting these pathways by different mechanisms could be promising in the management and prophylaxis against diabetic cardiomyopathy.

List of abbreviations

AMP	Adenosine monophosphate
HFpEF	heart failure with normal ejection fraction
HFrEF	heart failure with reduced ejection fraction
GLP-1	glucagon-like peptide 1
GLUT-4	glucose transporter type 4
eNOS	endothelium nitric oxidase enzyme
NO	Nitric oxide
IGF-1	insulin-like growth factor 1
ERK1/2	extracellular signal-regulated kinase 2
PI3K	phosphatidylinositol 3-kinase
RAAS	renin angiotensin aldosterone system
AGE	advanced glycation end products
TGF-β1	transforming growth factor beta 1
Akt	Protein kinase B

IRS	Insulin receptor substrate
ATP	Adenosine triphosphate
mTOR	mammalian target of rapamycin
FoxO1	fork head box-containing protein, O
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B
ROS	Reactive oxygen species
FFA	Free fatty acids
CD36	cluster of differentiation 36
DAG	diacylglycerol
PKC	protein kinase C
CGI-58	comparative gene identification 58
NADPH	nicotinamide adenine dinucleotide phosphate
RAGE	Receptor of advanced glycation end products
MAPK	mitogen-activated protein kinase
JAK	Janus kinase
TNF-α	tumor necrosing factor-α
IL	Interleukin
NLRP3	Interleukin 8
MCP-1	monocyte chemotactic protein 1
TLR-4	Tool lie receptor 4
NLRP3	Cryopyrin
Ag-II	Angiotensin II
AT	Angiotensin II receptor
MR	Mineralocorticoid receptor
PERK	protein kinase RNA-like endoplasmic reticulum kinases
Atg	autophagy related 1
Bcl-2	B-cell lymphoma 2
EDHF	endothelium-derived hyperpolarizing factor
ET-1	endothelin-1
AMPK	AMP-activated protein kinase
PPARs	peroxisome proliferators activated receptors
SGLT1	sodium glucose transporter-1
JNK	C-jun N-terminal kinase
Nrf2	nuclear factor erythroid-2- related factor 2

1. Overview:

Because of diabetes, heart failure rate is increasing at an alarming rate leading to a high incidence of morbidity and mortality. For diabetic patients, the clinical outcomes arising from heart failure are more serious than non-diabetic patients. The presence of myocardial dysfunction in diabetic cases occurs in the absence of conventional cardiac risks such as coronary artery diseases, valvular diseases, hyperlipidemia and hypertension. This will lead to a specific descriptive terminology known as "diabetic cardiomyopathy".

Diabetic cardiomyopathy is mainly characterized by clinical heart failure that accompanied by cardiac fibrosis with systolic and diastolic dysfunction in addition to mitochondrial dysfunction, metabolic dysfunction, increased oxidative stress, reduced nitric oxide level, elevation in the collagen formation, impaired calcium flow, inflammation and activation of renin angiotensin-aldosterone system. The possible Molecular mechanisms that linked to diabetic cardiomyopathy include abnormalities in adenosine monophosphate (AMP)-activated protein kinase, protein kinase C, microRNA, O-linked-N-acetylglucosamine and exosome pathways.

In its early stages diabetic cardiomyopathy includes a hidden subclinical period characterized by structural and functional abnormalities, including left ventricular (LV) hypertrophy, fibrosis and cell signaling abnormalities. These pathophysiological changes of cardiac fibrosis and stiffness and associated subclinical diastolic dysfunction often evolve to heart failure with normal ejection fraction (HFpEF) and eventual systolic dysfunction accompanied by heart failure with reduced ejection fraction (HFrEF).

2. Clinical aspects of diabetic cardiomyopathy

Epidemiological studies related to diabetes showed that the prevalence of heart failure in diabetic patients ranges from 19% to 26% (Ryden *et al.*, 2000; Thrainsdottir *et al.*, 2005). It was found that the incidence of heart failure was increased in both diabetic males and females which was found to be independent on obesity, hypertension, hyperlipidemia and coronary heart diseases (Kannel *et al.*, 1974). Another study confirmed that the incidence of heart failure in diabetic patients (39%) is higher than non-diabetic patients (23%) (Aronow *et al.*, 1999). Furthermore, it was observed that, there is a link between the increased level of glycated hemoglobin and the risk of heart failure where, an increase in the glycated hemoglobin level by 1% will lead to an increase in heart failure risk by 30% in type 1 diabetic patients and 8% in type II diabetic patients suggesting that hyperglycemia is promotor for heart failure (Lind *et al.*, 2011).

Risk factors for diabetic cardiomyopathy are hyperglycemia, insulin resistance and impaired cardiac insulin metabolic signaling are major abnormalities in diabetes, and they are involved in the development of diabetic cardiomyopathy (Fig 1 and 2) (Jia *et al.*, 2016).

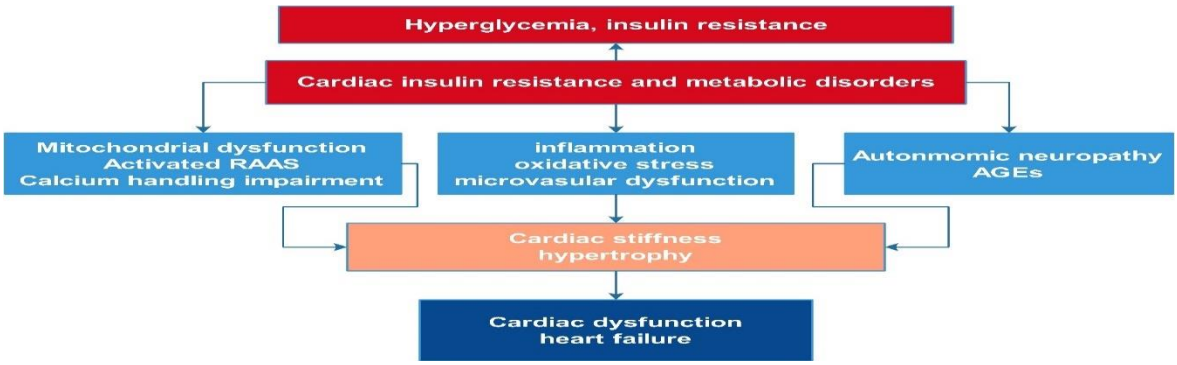

Fig. 1. Pathophysiological mechanisms of diabetic cardiomyopathy. Hyperglycemia, insulin resistance, and hyperinsulinemia induce cardiac insulin resistance and metabolic disorders that increase mitochondria dysfunction, oxidative stress, AGEs, impairment of mitochondria Ca2+ handling, inflammation, activation of RAAS, autonomic neuropathy, endoplasmic reticulum stress, cardiomyocyte death, as well as microvascular dysfunction. These pathophysiological abnormalities promote cardiac stiffness, hypertrophy, and fibrosis, resulting in cardiac diastolic dysfunction, systolic dysfunction, and heart failure. Abbreviations: AGEs, advanced glycation end-products; RAAS, renin-angiotensin-aldosterone system.

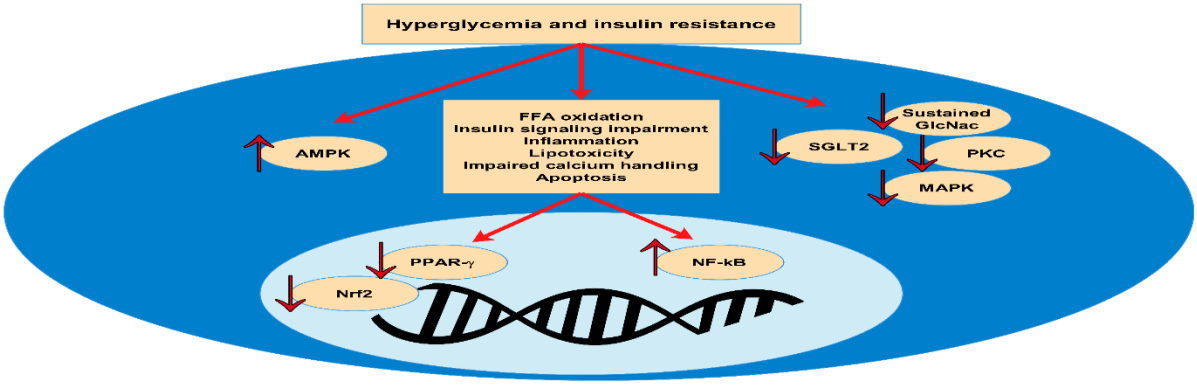

Fig. 2. The molecular proteins and signaling pathways in hyperglycemia- and insulin resistance diabetic cardiomyopathy. Increased PKC, MAPK, NF-κB, SGLT2, O -GlcNAc and CREM signaling, dysregulation of miRNA and exosomes, and reduction of AMPK, PPAR-γ and Nrf2 induce cardiac insulin resistance, subcellular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

3. Progression of diabetic cardiomyopathy to heart failure

Diabetic cardiomyopathy is asymptomatic in the early stages (Jia *et al.*, 2016). The earliest manifestations are left ventricular hypertrophy that is associated left ventricular compliance that is characterized by dysfunction in early diastolic filling, increased atrial filling, and prolonged isovolumetric relaxation (Jia *et al.*, 2016).The development of systolic dysfunction will lead to subsequent symptomatic heart failure.

The diastolic dysfunction that observed by magnetic resonance in rats showed its association with impaired cardiac metabolic signalling of insulin (Jia *et al.*, 2015). The cardiac abnormality is believed to be due to cardiomyocytes stiffness, cardiac fibrosis and cardiac hypertrophy (Fig. 1). A study carried by The Cardiovascular Health showed that the ventricular septal wall and the left ventricular wall was greater in diabetic patients compared to non-diabetic which led to impairment in cardiac functions (M. Lee *et al.*, 1997).

There is a remarkable epidemiological evidence that obesity, due to excessive carbohydrates intake and decreased exercise, is one of the most risk factors of diabetes and associated cardiac abnormalities. Changes in life style such as exercise, diet and weight control, smoking cessation and minimizing stress are efficacious approaches for the prophylaxis against diabetic cardiomyopathy. Sustained control of blood glucose level (BGL) reduces the chance of diabetic cardiomyopathy. A study carried by (Tate *et al.*, 2017) proved that normalization of BGL by insulin therapy reduced cardiac collagen formation the diastolic dysfunction in type 1 diabetic model in rats. Additionally, in a retrospective study of 10920 patients with type II diabetes, metformin administration is associated with low mortality risk in diabetic patients (Andersson *et al.*, 2010). Moreover, the beneficial effect on cardiovascular outcomes of glucagon-like peptide 1 (GLP-1) receptor agonist and sodium-glucose transporters was remarkable in type 2 diabetic patients (Jia *et al.*, 2016). In contrast, it was found that peroxisome proliferator activated receptor agonists and dipeptidyl peptidase 4 inhibitors increased the risk of heart failure in type 2 diabetes. Finally, treatment with two long acting GLP-1 receptor agonists significantly minimized the cardiovascular events and heart failure in high risk type 2 diabetic patients (MarsoBain *et al.*, 2016; MarsoDaniels *et al.*, 2016). These results suggest that the administration of these anti-diabetic drugs may have a role in lowering the risk of the development of cardiovascular disorders in type 2 diabetes.

4. Functional phenotype of diabetic cardiomyopathy

The first stage of diabetic cardiomyopathy is usually asymptomatic. It is characterized by an increase in collagen and fibrotic rates which lead to stiffness; a reduced early diastolic filling; arterial enlargement with increased early filling; elevated left ventricular end-diastolic pressure (Westermeier *et al.*, 2016). Many factors are contributed in the development of diabetic

cardiomyopathy including: hyperglycemia, increased free fatty acids level, cardiac insulin resistance, tissue inflammation, oxidative stress, activation of renin angiotensin-aldosterone system, sympathetic overstimulation, reduced activation of Ca^{++} (Fig.1) pump and insufficient sequestration of Ca^{++} in endoplasmic reticulum (Talukder *et al.*, 2008).

The second stage of diabetic cardiomyopathy is distinguished by left ventricular hypertrophy, advanced cardiac diastolic dysfunction, and the consequent appearance of clinical manifestations of heart failure (Jia *et al.*, 2016). Along with the development of diabetic cardiomyopathy, diastolic dysfunction and reduced cardiac function may exist with systolic dysfunction which may lead to a reduction in ejection fraction, enlargement in the left ventricles, shortened ejection period, and increased filling pressure with increased resistance to filling (Jia *et al.*, 2016). The mechanical defects in cardiac contraction is due to abnormalities in the expression of contractile and regulatory protein (Pollack *et al.*, 1986). Troponin phosphorylation is believed to contribute in the depression myocardial contractility, since cardiomyocytes contraction could be regulated by myosin light chain-2 and troponin I (Malhotra *et al.*, 1997).

The underlying mechanisms involved in diabetic cardiomyopathy in type 2 diabetes was mainly investigated in db/db mice, ob/ob mice, and diabetic patients (Jia *et al.*, 2016). The impact of type 1 diabetes on systolic and diastolic function is less clear. However, diastolic dysfunction was often observed in type 1 diabetes as well as type 2 diabetes (Kanamori *et al.*, 2015). In type diabetes the underlying mechanisms of cardiomyopathy are overlapped but in type 2 diabetes different molecular alterations are found (Holscher *et al.*, 2016). For example, cardiac hypertrophy was not observed and systolic functions was preserved in type 1 diabetes of Akita mice compared to control animals (Bugger *et al.*, 2008). The autophagy of cardiomyocytes was enhanced in type 1 diabetes while, it was suppressed in type 2 diabetes (Kanamori *et al.*, 2015). Therefore, different studies were essential in order to understand the differences in phenotypes and the underlying mechanisms that are associated with diabetic cardiomyopathy in both type 1 and type 2 diabetes.

In conclusion, the cardiac dysfunction in diabetic patients starts from subclinical cardiac abnormalities such as left ventricular fibrosis to reach observable diastolic dysfunction and eventually a reduced ejection fraction due to systolic dysfunction. Many non-invasive techniques are sued to detect any changes in the cardiac structure and function including echocardiography, computed tomography, and magnetic resonance imaging (Jia *et al.*, 2016). Moreover, other blood markers such as elevated levels of atrial natriuretic peptide and O -linked N-acetylglucosamine are important markers for the diagnosis of diabetic cardiomyopathy (Jia *et al.*, 2016).

5. Molecular mechanisms underlying diabetic cardiomyopathy

Cardiac structural abnormalities

The mechanism responsible for cardiomyocytes stiffness in the diabetic cardiomyopathy includes lowering of sarcoplasmic Ca++ pump due to the decreased glucose transporter type 4 (GLUT4) activity with accompanied decrease in glucose uptake (Jia *et al.*, 2016). Moreover, the abnormal insulin metabolic signalling will decrease insulin-mediated endothelium nitric oxidase enzyme (eNOS) activity which lead to a decrease in nitric oxide (NO) formation leading to an increase in intracellular Ca++ with concurrent decrease in Ca uptake by sarcoplasmic reticulum (Jia *et al.*, 2016). Additionally, the decrease in NO availability will decrease the phosphorylation of titin increasing the expression of stiff titin isoform N2B/N2BA which will increase the cardiac stiffness and impair the cardiac relaxation (Jia *et al.*, 2016).

Other relevant abnormalities in diabetes include hyperglycemia, insulin resistance and oxidative stress. This will increase the expression cardiomyocyte hypertrophic genes including insulin-like growth factor 1 (IGF-1) receptor, and B type natriuretic peptide (Rosenkranz *et al.*, 2003). Moreover, IGF-1 could promote cardiac hypertrophy through different signaling pathways such as extracellular signal-regulated kinase 2 (Erk1/2) and phosphatidylinositol 3-kinase (PI3K) pathways (Sundgren *et al.*, 2003).

The changes in cardiac architecture in diabetic cardiomyopathy include stiff collagen deposition, cardiac interstitial fibrosis, abolition of cardiac fibrils, perivascular fibrosis, thickened small coronary blood vessels and sclerosis of microvascular coronary blood vessels (Mytas *et al.*, 2009; J. Wang *et al.*, 2006). Activation of the renin angiotensin aldosterone system (RAAS) and advanced glycation end products (AGE)-mediated signaling in addition to hyperglycemia could lead to the activation of transforming growth factor beta 1 (TGF-β1) pathway and extracellular matrix degradation (Bando *et al.*, 2014). Many biomarkers could be clinically for diagnosis of cardiomyopathy such as inflammatory cytokines, connective tissue growth factors, galectin-3 and metalloproteinases (Passino *et al.*, 2015). So, increased fibronectin level, collagen content in association with interstitial fibrosis are characteristic sign for diabetic cardiomyopathy that occur due to reduce NO availability, increased oxidative stress, impaired insulin metabolic signaling and elevated TGF-β (Jia *et al.*, 2015).

Cardiac insulin resistance and diabetic cardiomyopathy

Cardiac insulin signaling controls many cellular metabolic processes via controlling protein synthesis, glucose utilization, and cell survival. As in skeletal muscles and other body tissues, the glucose transport to the cardiac tissue is mediated by GLUT4. When insulin binds to its receptor, it activates insulin signaling/docking molecule insulin receptor substrate (IRS)-1/2 and subsequent stimulation of PI3K/protein kinase B (Akt) leading to stimulation of GLUT4 translocation to the cell membrane (Jia *et al.*, 2016). Furthermore, myocardial insulin activity promotes eNOS activation and NO

formation which is necessary for the optimal coronary microvascular blood flow and cardiac functions (Jia *et al.*, 2016; Jia *et al.*, 2015). Lack of insulin receptor in myocardium decreases the glucose uptake, which lead to an increase in cardiac reactive oxygen species (ROS) formation, which cause mitochondrial dysfunction (Bugger *et al.*, 2012; Qi *et al.*, 2013). Furthermore, adenosine triphosphate (ATP) content was reduced in IRS-1/2 knockout cardiomyocytes which impairs cardiac metabolism, that is accompanied with increased fibrosis and high incidence of cardiac failure (Bugger *et al.*, 2012; Qi *et al.*, 2013).

The E3 ubiquitin ligase, mitsugumin 53, may play an essential negative role in maintaining insulin signaling (Song *et al.*, 2013). Elevated cardiac MG53 protein levels were linked to the increased degradation of insulin receptor. Additionally, the insulin signaling was diminished and fibrosis increased after overexpression of MG53 in cardiomyocytes (F. Liu *et al.*, 2015) suggesting that decreasing cardiac levels of MG53 may be a potential therapeutic target in the prevention of diabetic cardiomyopathy and progression to heart failure.

The cardiac insulin metabolism can may be impaired by risk factors such as obesity and RAAS abnormalities. This occur through enhanced activation of mammalian target of rapamycin (mTOR) signaling pathway (Jia *et al.*, 2016), which increases the phosphorylation of serine with reduced tyrosine phosphorylation of IRS-1/2 leading to ana impairment in PI3K engagement and Akt/eNOS induction. This will impair NO formation leading to further impairment in coronary blood vessels relaxation which will decrease the delivery of glucose to cardiac tissue (Jones *et al.*, 2006; Vincent *et al.*, 2004; Vincent *et al.*, 2006; Vollus *et al.*, 2007). Additionally, NO impairment will activate the collagen cross-linking promoting cardiac fibrosis and stiffness (Bertoni *et al.*, 2006).

Tumor necrosing factor alpha (TNF-α) is a pro-inflammatory cytokine, which was proved to promote cardiac insulin resistance through promoting the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun N terminal kinase (JNK). NF- κB and JNK induce phosphorylation of IRS-1 (Jia *et al.*, 2016). Activation of fork head box-containing protein, O subfamily (FoxO1) directly lead to insulin resistance through regulating IRS1 signaling and decreasing PI3K/Akt signaling (Battiprolu *et al.*, 2012). Furthermore, heart failure was markedly decreased after deletion of cardiac FoxO1 in animals (Qi *et al.*, 2015). Therefore, FoxO1 may provide an interesting therapeutic strategy for treating diabetic cardiomyopathy (Qi *et al.*, 2015).

Decreased flexibility in substrate utilization in diabetic cardiomyopathy

In normal physiological conditions, the heart flexibly utilizes energy from various substrates including free fatty acids, glucose, lactate, ketone bodies and some amino acids. This essential for the production of ATP as a source of energy essential for normal cardiac function (Jia *et al.*, 2016). About 95% of ATP

is produced by oxidative phosphorylation in the mitochondria of cardiac cell, whereas, the remaining 5% of the remaining ATP is formed glucose or lactate in cardiac tissue (Guo *et al.*, 2017). Mitochondrial volume represents about 20-30% of cardiomyocytes total volume (Jia *et al.*, 2016). The insulin resistance reduces the cardiac ability for glucose uptake leading to switching to the free fatty acids utilization (Guo *et al.*, 2017). As a result, the oxidative phosphorylation is impaired and the production of reactive oxygen species (ROS) is increased. Due to the limited anti-oxidant capacity in the cardiac tissue, this will lead to the destruction of NO and reducing the its bioavailability (MarsoBain *et al.*, 2016).

The role of abnormal FFA metabolism in diabetic cardiomyopathy

The diabetic cardiomyopathy is also increased by the concurrent increase in free fatty acids (FFA) released form adipose tissue and the elevated free fatty acids capacity of myocytes sarcolemma (Guo *et al.*, 2017). The free fatty acids uptake is promoted by the cluster of differentiation 36 (CD36) which is a protein that located in the cell membrane of myocytes and believed to be abundant in diabetic cardiomyopathy (T. W. Lee *et al.*, 2017). This will lead to an elevation in the free fatty acids uptake rate in diabetics' heart (T. W. Lee *et al.*, 2017). Additionally, CD36 is participated in adenosine monophosphate activated (AMPK) role in cardiomyocytes uptake of free fatty acids. That was proved in CD36-kockout mice, where, the uptake of free fatty acids was reduced by 70% in their hearts (Habets *et al.*, 2007). AMPK activation seems to be responsible for glucose uptake and glycolysis in diabetic cardiomyocytes (Zou *et al.*, 2013). However, its activation will lead to an accompanied increase in the free fatty acids uptake and accumulation of triglycerides which in turn reduces the glucose utilization that is common in diabetic cardiomyopathy (Finck *et al.*, 2002).

The diabetic cardiomyopathy is aggravated by many lipid metabolites such as diacylglycerol (DAG) that impair the insulin signaling mechanisms. In obese, the decrease in insulin sensitivity is believed to be associated with elevated DAG and increased protein kinase C (PKC) activity. The endoplasmic reticulum stress is increased by DAG (Samuel *et al.*, 2016). Moreover, increased DAG will lower the formation of NO (Atkinson *et al.*, 2003).

The triglycerides metabolism by lipase enzyme is regulated by a lipid droplet-associated protein known as comparative gene identification 58 (CGI-58) (Cantley *et al.*, 2013). The knockout of CGI-58 gene prevented the accumulation of DAG at plasma membrane in addition to inhibiting PKC activity (Jornayvaz *et al.*, 2011).

Meanwhile, another lipid metabolite known as ceramide can activate PKC leading to subsequent inhibition of insulin metabolic Akt/PKB signaling, emaciating the translocation of GLUT4 and insulin mediated glucose uptake by diabetic cardiomyocytes (Atkinson *et al.*, 2003).

Mitochondrial dysfunction in the genesis of diabetic cardiomyopathy

The mitochondrial dysfunction could be involved in the development of cardiomyopathy and heart failure in diabetics (Kim *et al.*, 2008). Over 90% of ATP is formed by mitochondrial oxidative phosphorylation in cardiomyocytes, but, in diabetics the production of ATP is switched from glucose utilization into free fatty acids utilization by mitochondria (Jia *et al.*, 2016). This will lead to subsequent elevation in the generation of ROS due to impaired oxidative phosphorylation. Furthermore, cell death is promoted by respiratory dysfunction in mitochondria due to changed mitochondrial Ca^{++} handling (Anderson *et al.*, 2009). Cardiomyocytes autophagy and cardiac cell death may occur due to increased Ca^{++} overload induced by metabolic stress that lead to excessive opening of mitochondrial transition pores (Anderson *et al.*, 2011).

Mitochondrial oxidative stress in the pathogenesis of diabetic cardiomyopathy.

Cardiac insulin resistance and its progression to cardiomyopathy is promoted by oxidative stress. Mitochondrial oxygen metabolism at complex I and II within electron transport chain usually results in the formation of several byproducts such as ROS (Jia *et al.*, 2016). Under normal physiological conditions, the major electrochemical proton gradient is used for synthetization of ATP (Jia *et al.*, 2016).

Insulin resistance and hyperglycemia activate the influx of NADH into the respiratory chain of mitochondria which may result in the hyperpolarization of the inner membrane of mitochondria, electron transport inhibition in complex III and excessive production of ROS (Teshima *et al.*, 2014). Another source of ROS in cardiac tissue is the nicotinamide adenine dinucleotide phosphate (NADPH). The increased activity of NADPH oxidase was previously recorded in cardiac insulin resistance and obesity (Jia *et al.*, 2018). Additionally, the cardiac fibrosis could be enhanced by RAAS-mediated NADPH oxidase activity through activating TGF-β1-Smad 2/3 pathway (S. J. Lee *et al.*, 2009; Murdoch *et al.*, 2014).

Increasing the activity of xanthine oxidase and microsomal P-450 activity is considered and important source of ROS in diabetic cardiomyopathy. They are involved in several mechanisms including the elevated expression of AGEs receptor, increased PKC signalling and the inhibition of eNOS and prostacyclin synthase activity (Giacco *et al.*, 2010).

Role of AGEs and RAGE in the pathophysiology of diabetic cardiomyopathy

Hyperglycemia is contributed in the accumulation of AGEs and the subsequent alteration in the myocardial structure. This occurs due to several mechanisms such as lips and protein oxidation, nonenzymatic glycation, production of myocardial collagen and fibronectin in addition to cross-linking of connective tissue and fibrosis (Jia *et al.*, 2016). The AGE-induced cross-linking of connective tissue of extracellular matrix stimulates the fibrotic process leading to

impaired passive relaxation (Jia *et al.*, 2016). Moreover, AGEs may promote cardiac structural changes and alter the energetics of myocardium through binding to Receptor of advanced glycation end products (RAGE) (Jia et al., 2016). This reaction stimulates the formation of pro-inflammatory responses and the production of connective tissue which is mediated by mitogen-activated protein kinase (MAPK) and Janus kinase (JAK) pathways (Jia *et al.*, 2016). Also, as mentioned before, the AGEs may elevate the production of ROS and TGF-β1-Smad 2/3 pathway activation which will lead to fibrosis and production of connective tissue.

Impaired mitochondrial Ca2+ handling in diabetic cardiomyopathy

The cytosolic Ca^{++} regulates several physiological and biochemical functions including regulation of cellular metabolism, muscle contraction and cell signalling. In cardiac contraction, the Ca^{++} reaches the cytoplasm through L-type voltage sensitive channels of sarcolemma after depolarization. This will trigger the release of Ca^{++} from the sarcoplasmic reticulum which binds to troponin C protein and induce the contraction of myofibrils (Kanaporis *et al.*, 2017; W. Liu *et al.*, 2017). During cardiac relaxation, Ca^{++} is re-uptaken by sarcoplasmic reticulum while the remaining of Ca^{++} is pumped out actively by the aid of Na^{+}/Ca^{++} exchanger or by Ca^{++} pumps in the plasma membrane (Kanaporis *et al.*, 2017). In diabetic cardiomyopathy, the handling of Ca^{++} transport by Ca^{++} membrane pump and the Na^{+}/ Ca^{++} exchanger is impaired which may lead to the prolongation of diastolic relaxation (Jia *et al.*, 2016). Additionally, the accumulation of intracellular Ca^{++} will prolong the decay of intracellular Ca^{++}, slow the Ca^{++} transients and impair the uptake of Ca^{++} by the sarcoplasmic reticulum (Belke *et al.*, 2004; Ye *et al.*, 2004).

Inflammation as an instigator of diabetic cardiomyopathy

A maladaptive inflammatory response was indicated in diabetic cardiomyopathy development. This process involves many inflammatory cells including mast cells, dendritic cells, macrophages, eosinophils and neutrophils (Jia *et al.*, 2016). The expression of several inflammatory mediators such as tumor necrosing factor-α (TNF-α), interlukin-6 (IL-6), interlukin-8 (IL-8), vascular cell adhesion molecule and monocyte chemotactic protein 1 (MCP-1) was proved to increase in diabetic cardiomyopathy. All these inflammatory mediators may participate in oxidative stress and cardiac fibrosis which is associated with diastolic dysfunction (Jia *et al.*, 2016). Toll like receptor 4 (TLR-4) have a role in increasing the expression of these cytokines and innate immune responses through triggering NF-κB activity (Uchimura *et al.*, 2014). This inflammatory response could occur in different cardiac cells including endothelial coronary cells, fibroblasts and cardiomyocytes.

The NLRP3 inflammasome could be activated due to impaired insulin metabolic signalling which is associated with activation of procaspase-1 (Pal *et al.*, 2017). the activated caspase-1 along with other

cytokines such as IL-1β and IL-18 may enhance other inflammatory pathways involving ROS, chemokines and NF-κB. the increased ROS and coronary monocytes/macrophages migration will enhance the polarization of these inflammatory cells into proinflammatory M1 phenotypes. This was proves by many studies which stated that in diabetic cardiomyopathy the level of proinflammatory M1 phenotype was elevated while the antiinflammatory M2 phenotype was depressed (Jia *et al.*, 2015).

Activated RAAS in the genesis of diabetic cardiomyopathy

Due to hyperglycemia and insulin resistance, the activation of systemic and tissue RAAS is increased which play an important role in diabetic cardiomyopathy and myocardial fibrosis (Jia *et al.*, 2015). There is a correlation between serum levels of angiotensin II (Ag-II) and plasma glucose level in insulin resistance (Xu *et al.*, 2013). Additionally, the expression of the proinflammatory Ag-II receptor 1 (AT-1) is increased while the expression of the anti-inflammatory Ag-II receptor 2 (AT-2) is repressed in diabetics (Giacchetti *et al.*, 2005). Many studies prove that insulin resistance, the activated RAAS play a role in diabetic cardiomyopathy through increasing mineralocorticoids systemic level with concurrent overexpression of tissue mineralocorticoid receptor (MR) (Baudrand *et al.*, 2016; Miller, 1999; Miller *et al.*, 1996). The inhibition of MR signalling pathway minimizes mortality and morbidity in diabetic patients. Moreover, RAAS stimulation could activate mTOR/S6K1 signalling which may have a role in systemic and cardiac insulin resistance induction (Kim *et al.*, 2012).

Endoplasmic reticulum stress and increased cell death in diabetic cardiomyopathy

The myocardial endoplasmic reticulum function and stress is impaired in diabetic cardiomyopathy due to oxidative stress, release of proinflammatory mediators, lipotoxicity and the cumulation of misfolded proteins (Jia *et al.*, 2016). Meanwhile, increased levels of unfolded protein and their degradation inhibits the protein synthesis and stimulate apoptotic processes and autophagy (Jia *et al.*, 2016) which is a main risk factor in developing cardiac myopathy. In diabetic cardiomyopathy, the autophagy that is induced by endoplasmic stress is controlled by several pathways including Ca^{++}-dependent pathway, protein kinase RNA-like endoplasmic reticulum kinases (PERK) pathway, AMPK, mTOR and silent information regulator (sirt) pathways (Jia *et al.*, 2016).

mTORC1 has been found to regulate autophagy by depressing the autophagy related 1 (Atg1)–Atg13–Atg101/FIP200 (FAK family interacting protein of 200 kDa) complex. Thus, the initiation of autophagy process is facilitated by inhibition of mTORC1 (L. Yang *et al.*, 2015). The inositol requiring enzyme 1 arm of ER stress results in the activation of JNK and elevated phosphorylation of B-cell lymphoma (Bcl-2), which encourages its dissociation from Beclin-1 (L. Yang *et al.*, 2015). Thus, enhanced mTOR may serve as a meeting

point for abnormalities involving the relationship between endoplasmic reticulum stress and autophagy in diabetic cardiomyopathy. However, in diabetic cardiomyopathy, dysregulation of autophagy impairs cardiomyocyte auto-phagosome and lysosome fusion (Xie *et al.*, 2011). One study confirmed that treatment with metformin could increase AMPK activity with restoring autophagic activity, and inhibiting cardiomyocyte apoptosis. This may be performed by interfering with the Bcl-2 and Beclin1 complex in diabetic cardiac tissue (Xie *et al.*, 2011).

Microvascular dysfunction in diabetic cardiomyopathy

Diabetic cardiomyopathy is defined as a cardiomyopathy that occur in the absence of notable coronary artery disease. however, diabetic cardiomyopathy could be accompanied with coronary vascular abnormalities that may affect the coronary blood flow. Decreasing coronary supply and myocardial perfusion will result in impairing the ventricular function which leads to subsequent clinical outcomes (Sandesara *et al.*, 2018). The administration of MR antagonist minimized the occurrence of any cardiovascular disease in patients with type II diabetes through improving the coronary blood flow (Garg *et al.*, 2015). Several structural and functional abnormalities in coronary blood vessels may occur in diabetics; luminal obstruction, infiltration of inflammatory cells and vascular fibrosis as structural changes while functional changes include dysfunction endothelial cells dysfunction, impaired vasorelaxation and vasoconstriction, diminished cardiac perfusion (Shome *et al.*, 2017). The endothelial cells of the coronary blood vessels secrete several vasoactive substances such as NO, prostacyclin, endothelium-derived hyperpolarizing factor (EDHF). These factors play an essential vasodilator role (Vincent *et al.*, 2004). In early stages of diabetes, the NO-induced vasodilation is impaired but the normal vascular function is preserved due to enhanced EDHF activity (Vincent *et al.*, 2004). However, the activity of both factors is affected at last stages leading to significant vascular dysfunction (Vincent *et al.*, 2004). High plasma concentration of endothelin-1 (ET-1) was recorded with concurrent diminution in eNOS production in diabetic cardiomyopathy (Widyantoro *et al.*, 2010).

Impaired AMPK activation in diabetic cardiomyopathy

The AMP-activated protein kinase (AMPK) is an essential regulator of cellular homeostasis (Jia *et al.*, 2016). The translocation of GLUT4 and expression is enhanced by cellular stress and elevated AMP/ATP ratio. This will increase the biogenesis of mitochondria leading to FFA oxidation and glycolysis (Zou *et al.*, 2013). The activation of AMPK elevates the glucose uptake and reduce mTOR signalling and the synthesis of lipids and proteins (Abdel Malik *et al.*, 2017). Therefore, enhancing the AMPK activity prevents the progression of diabetic cardiomyopathy. For this reason, AMPK is considered as a promising target for the treatment and prevention of diabetic cardiomyopathy.

Alterations in activation of cardiac PPARs

The peroxisome proliferators activated receptors (PPARs) are expressed in different isoforms (α, β/δ and γ). They play a key role in lipid metabolism and energy hemostasis. Additionally, they exert different roles related to metabolism such as oxidative stress and inflammation (Jia *et al.*, 2016). PPAR-α is highly expressed in the cardiac tissue and its activation affects FFA uptake their oxidation (Jia *et al.*, 2016). PPAR-α is essential in regulating the assembly of lipoprotein and regulates oxidant and antioxidant defenses (T. I. Lee *et al.*, 2013). the increased activity of cardiac PPAR-α decreases the Ca++ uptake by sarcoplasmic reticulum which may lead to left ventricular hypertrophy, systolic dysfunction and elevated expression of B-type atrial natriuretic peptide (Finck *et al.*, 2002).

Conversely, deletion of cardiac PPAR-α prevents fasting-induced expression of FFA metabolic genes and induces a switch from FFA to glucose utilization (Leone *et al.*, 1999). The expression of PPAR-α is reduced in diabetic cardiomyopathy due to extensive exposure to elevated FFA. This will lead to a decrease in the FFA oxidation and accumulation of fats which may cause further depression in cardiac functions (Young *et al.*, 2001; Zhou *et al.*, 2000). However, studies performed on human type-II diabetic patients showed no significant alteration in PPAR-α expression (Razeghi *et al.*, 2002). Further, diminution in PPAR-α in advanced disease may have maladaptive significances in standings of cardiac metabolism, including glucotoxicity and abnormalities in cardiac functions.

The role of PPAR-α in cardiac dysfunction development in diabetes has been insufficiently evaluated and needs further investigations. Similarly, PPAR-β/δ isoforms are expressed in cardiac tissue and regulate the expression of transcriptional gene and the metabolism of FFA (Cheng *et al.*, 2004). Enhanced PPAR-β/δ signaling encourages the FFA utilization where, the deletion of PPAR- β/δ reduces the expression of FFA oxidative gene and FFA oxidation (Cheng *et al.*, 2004). Additionally, PPAR-γ has an anti-hypertrophic and antiinflammatory roles in heart (Jia *et al.*, 2016). Activation of PPAR-γ enhances the glucose uptake by cardiac tissue and improves the cardiomyocyte insulin sensitivity (Jia *et al.*, 2016). Thus, PPAR-γ may be beneficial in maintaining glucose and FFA metabolism and cardiac function.

SGLT2 abnormalities and potential cardiac benefits of inhibition of this transporter

Glucose is actively absorbed from the gastrointestinal lumen into its epithelium primarily by sodium glucose transporter-1 (SGLT1), which is highly expressed in the membrane of enterocytes (Lehrke *et al.*, 2017; Pham *et al.*, 2017). In case of hyperglycemia and insulin resistance, the increased expression of SGLT1 and glucose transporters (GLUT1, GLUT2 and GLUT5) in addition to increased activity of sucrase, maltase and lactase, will increase the absorption of glucose and fructose through the intestinal mucosa (Lehrke *et al.*, 2017; Pham *et al.*, 2017). SGLT2 is primarily

expressed in the epithelial membrane of S1 segment of the proximal convoluted tubules in the kidneys (Lehrke *et al.*, 2017; Pham *et al.*, 2017). In diabetes, the expression of SGLT2 is markedly elevated in both humans, mice and rats (Sabolic *et al.*, 2012; von Lewinski *et al.*, 2010) which is related to the elevated BGL, increased glomerular filtration and reabsorption (Lytvyn *et al.*, 2017). Conversely, the inhibition of SGLT2 leads to osmotic diuresis with increased sodium loss which lead to water loss and reduced blood pressure. All these mechanisms may alleviate diabetic cardiomyopathy and heart failure. Furthermore, treatment with SGLT2 inhibitors could shift the cellular metabolism from glucose to free fatty acids oxidation. This will increase the formation of ketone body, β-hydroxybutyrate, which is and efficient energy source for cardiac metabolism (Zinman *et al.*, 2015).

PKC activation promotes development of diabetic cardiomyopathy

Protein kinase C signaling pathways are activated in diabetic cardiomyopathy due to hyperglycemia and insulin resistance. Furthermore, the PKC activity is promoted by inflammation, oxidative stress and enhanced RAAS activity. The PKC is found in different isoforms where, approximately fifteen isoforms where discovered in humans. These isoforms can be classified into 3 major subfamilies based on their signaling pathway and their mode of activation (Li *et al.*, 2014). The main PKC isoform found to be involved in the development of diabetic cardiomyopathy are PKCα, PKCβ, PKCε, PKCθ, and PKCδ (Li *et al.*, 2014). For example, PKC β2 was discovered to control hyperglycemia-induced cardiomyopathy in diabetic rats through alterations in insulin metabolic Akt/eNOS signaling pathway (Lei *et al.*, 2013). moreover, the cardiac hypertrophy and fibrosis was reported to be improved in transgenic mouse model of diabetic cardiomyopathy subjected to targeted inhibition of PKCβ2 (Wakasaki *et al.*, 1997).

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

The activation of MAPK activation was found to be involved in the pathogenesis of diabetic cardiomyopathy and heart failure. There are three major MAPK subfamilies that regulate cardiac growth and remodeling (Jia *et al.*, 2016). These subfamilies are Erk1/2, p38 MAPK and JNKs. In streptozotocin-insulted diabetic models, the phosphorylation and activation of Erk 1/2 and p38 was elevated (Strniskova *et al.*, 2003). Moreover, it was demonstrated that insulin resistance-induced cardiac abnormalities were associated with increased Erk 1/2 signalling (H. Zhang *et al.*, 2001). C-jun N-terminal kinase (JNK) is mainly activated by inflammatory cytokine and oxidative stress which is contributed to endoplasmic reticulum stress and cardiac fibrosis. In contrast, the inhibition of JNK activation prevented the cardiac inflammation and apoptosis in diabetics (Y. Wang *et al.*, 2014). JNK activation was reported to increase the cardiomyocyte apoptosis within a week in rodent diabetic model (Gurusamy *et al.*, 2004).

Role of NF-κB activation in the genesis of diabetic cardiomyopathy

The NF-κB is a major transcription factors that control proinflammatory cytokines expression and cell survival (J. Yang *et al.*, 2009) thus, it is contributed to cardiac dysfunction in diabetic hearts. In non-stimulated cells, the NF-κB was found in cytoplasm however, after stimulation, IκB is phosphorylated and p50/p60 subunits are translocated to nucleus and bind with the κB (J. Yang *et al.*, 2009). in diabetes, the NF-κB is activated by several factors such as ROS, AGEs and activated cardiac tissue RAAS which will result in subsequent maladaptive immune responses and the release of pro-inflammatory cytokines such as IL-6 and TNFα (Jia *et al.*, 2016).

Activated NF-κB in diabetic heart was associated with increased generation of ROS due to increased NADPH oxidase activity (Mariappan *et al.*, 2010) which lead to reduction in NO bioavailability. Inhibition of NF-κB with pyrrolidine dithiocarbamate was shown to improve structural integrity of mitochondria and prevent the oxidative stress, leading to an increase in ATP synthesis and NO bioavailability which, restores cardiac function in diabetic animals **(Mariappan *et al.*, 2010).**

Abnormalities of Nrf2 related antioxidant actions in diabetic cardiomyopathy

The nuclear factor erythroid-2- related factor 2 (Nrf2) is a leucine zipper protein that stimulate the expression of antioxidant proteins such as HO-1 in response to oxidative stress (Fig 2). Nrf2 is regulated by keap1 as an inhibitor that targets Nrf2 for degradation thus reducing the levels of Nrf2 **(Niture *et al.*, 2014).** Under oxidative stress, Nrf2 and its regulators are subjected to different modifications that cause Nrf2 dissociation from Keap1.

Free Nrf2 can then bind to small Maf proteins transcription factors in the nucleus to initiate the transcription process **(Niture *et al.*, 2014).** Hyperglycemia and insulin resistance suppress the expression of Nrf2 and its activity through an Erk 1/2 mediated pathway in cardiomyocytes **(Tan *et al.*, 2011).** Restoration of Nrf2 activity prevents diabetes-associated complications such as inflammatory reactions, fibrosis, and cardiac dysfunction **(Z. Zhang *et al.*, 2014).** This could provide a potential strategy for the prophylaxis against diabetic cardiomyopathy.

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