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Metabolic Dysfunction in Diabetic Cardiomyopathy

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Abstract

Diabetic cardiomyopathy (DCM) is defined as cardiac disease independent of vascular complications during diabetes. The number of new cases of DCM is rising at epidemic rates in proportion to newly diagnosed cases of diabetes mellitus (DM) throughout the world. DCM is a heart failure syndrome found in diabetic patients that is characterized by left ventricular hypertrophy and reduced diastolic function, with or without concurrent systolic dysfunction, occurring in the absence of hypertension and coronary artery disease. DCM and other diabetic complications are caused in part by elevations in blood glucose and lipids, characteristic of DM. Although there are pathological consequences to hyperglycemia and hyperlipidemia, the combination of the two metabolic abnormalities potentiates the severity of diabetic complications. A natural competition exists between glucose and fatty acid metabolism in the heart that is regulated by allosteric and feedback control and transcriptional modulation of key limiting enzymes. Inhibition of these glycolytic enzymes not only controls flux of substrate through the glycolytic pathway, but also leads to the diversion of glycolytic intermediate substrate through pathological pathways, which mediate the onset of diabetic complications. The present review describes the limiting steps involved in the development of these pathological pathways and the factors involved in the regulation of these limiting steps. Additionally, therapeutic options with demonstrated or postulated effects on DCM are described.

Diabetes mellitus

Diabetes mellitus (DM) is a global health epidemic whose rates have risen dramatically and are predicted to continue to rise during the next 20 years. It is estimated that 18.1 million people (8.0% of the adult population) in the United States have diagnosed DM, with another 7.1 million individuals having undiagnosed DM [1]. Similarly concerning is the 36.8% of the adult population who have abnormal fasting glucose levels, indicating clinical

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prediabetes. Type 2 DM (T2D) is particularly epidemic due to the rising rates of obesity throughout the world. Over one billion people worldwide are overweight (BMI >25 and <29.9) or obese (BMI >30) [2]. The projected obesity prevalence globally is 8.0% for men and 12.3% for women in 2010. DM is expected to rise worldwide from 175 million in 2000 to 353 million by 2030, creating a tremendous healthcare and financial burden [3]. The United States, with an overweight and obesity prevalence of 67.3% for adults older than twenty, is predicted to be the forerunner of the DM epidemic, increasing prevalence from 8.8% in 2000 to 11.2% by 2030 [1, 3].

Diabetes mellitus consists of several metabolic conditions in which there is a dysfunction in the cell's ability to transport and utilize glucose. Type 1 DM (T1D), formerly called insulin dependent or juvenile diabetes, is caused by T lymphocyte-mediated autoimmune destruction of the pancreatic β-cells, resulting in insufficient insulin production and corresponding decrease in glucose utilization [4]. The etiology of type 2 DM (T2D), formerly called insulin independent or adult-onset diabetes, results from an insulin resistance that instigates hypertrophy of the β-cell to compensate, resulting in hyperinsulinemia leading to eventual insulin resistance [5, 6]. Progressive decompensatory failure of the β-cells in T2D decreases the amount of insulin produced. The end result is a decreased level of serum insulin, which is insufficient to overcome the developed insulin resistance. These pathophysiological changes lead to elevated blood glucose levels (hyperglycemia) and impaired cellular glycolysis and pyruvate oxidation [7]. Chronic hyperglycemia can result in numerous comorbidities, including kidney failure, nerve damage, retinopathy, peripheral vascular disease and cardiac dysfunction/failure [8]. The mechanisms causing these comorbidities, particularly cardiac dysfunction, include increased levels of advanced glycation end products, mitochondrial dysfunction, enhanced oxidative stress, altered cell metabolic function and altered calcium homeostasis [8-10].

Cardiovascular and cardiomyocyte dysfunction in DM

Cardiovascular disease (CVD) resulted in one out of every three deaths in the United States in 2008, making it the leading cause of death often resulting from other medical conditions, including hypertension, alcoholism, obesity, and diabetes [1]. Additionally, heart disease death rates among adult diabetics is 2-4 times more likely than adults without DM and 68% of adults with DM older than 65 years die of some form of heart disease [11]. The significance of DM has especially increasing significance in women, as females with diabetes have a five times greater incidence of heart diseases than their non-diabetic counterparts, compared to the two fold increase in heart disease observed in diabetic versus non-diabetic men [12]. This discordance may be attributable to the intrinsic difference in the myocardium and/or sex hormonal and neurohormonal differences, but more gender specific studies are needed to fully describe the differences in mechanisms [13]. One secondary CVD is diabetic cardiomyopathy (DCM). The early stages of DCM involve observable left ventricular hypertrophy (LVH), which along with myocardial remodeling, causes abnormal left ventricle (LV) filling and diastolic dysfunction [14]. The left ventricular diastolic dysfunction (LVDD) is detectable via echocardiography [15]. Progression of DCM can lead to systolic dysfunction, which may be unrecognized in its early stages due to compensatory mechanisms preserving a normal ejection fraction in these individuals [14]. Functional alterations *in vivo* include decreased fractional shortening, decreased ventricular filling, decreased ventricular ejection fraction, increased ventricular wall stiffness and increased pre-ejection time [8]. This leads to abnormal relaxation, including increased isovolumetric relaxation time and impaired diastole [16].

Gross differentiation of T1D and T2D cardiomypathy

Diabetic cardiomyopathy has been largely considered as a single condition equally affecting all diabetic individuals. However, in recent years there has been increasing work utilizing animal models to differentiate the mechanisms and effects of DCM caused by T1D verses T2D. In general, studies have found that although systolic dysfunction is present in both, it is more prevalent in type 1 DCM [17]. Type 2 DCM models have been associated with a larger amount of preclinical changes than type 1 DCM, including a greater impairment of ventricular filling, resulting in more prevalent diastolic dysfunction [15, 18]. On gross pathological analyses of the myocardium, a newer finding has suggested that T2D patients have a significantly higher level of myocardial steatosis preceding and possibly contributing to the early diastolic dysfunction [19, 20]. Although the exact mechanisms for differences in DCM presentation of T1D and T2D is unknown, one possible explanation involves insulin resistance that shows reduced protective effects to ischemic/reperfusion [21, 22]. Other factors with a greater association with T2D such as hypertriglyceridemia and reactive oxygen species (ROS) due to augmented fatty acid metabolism have also been implicated in functional cardiomyocyte alterations [19, 20, 23]. Additional studies are warranted to elucidate and categorize type 1 verses type 2 diabetic cardiomyopathy.

Animal models of type 1 and type 2 diabetic cardiomyopathy

Increasing efforts in recent years have been placed in determining the mechanisms of DCM at the cardiomyocyte level. Aiding in this process, several rodent models have been developed to mirror the pathogenesis of T1D and T2D and the effects on the myocardium. Models referred to in this review will be briefly summarized. T1D is induced in a murine model by injection of streptozotocin (STZ) into the animal. Streptozotocin is a glucosamine-nitrosurea antibiotic that selectively targets and destroys pancreatic β-cells, resulting in hypoinsulineaemia and hyperglycemia. There are two methods of inducing T1D with STZ: injected singly at high concentrations, STZ preferentially accumulates in and kills pancreatic β-cells or given in multiple low doses an inflammatory response occurs in the β-cells, leading to lymphocytic infiltrates and cell death [24]. Either mechanism has been shown to effectively model the autoimmune T cell-mediated destruction and hypoinsulinemia observed in human T1D [15, 25]. The T2D model widely utilized is the Zucker fatty rat (ZFR), which displays an obesity-related (*fa*) trait resulting from leptin receptor genetic mutation [26]. The ZFR male homozygous (*fa/fa*) rat develops obesity and insulin independent diabetes (T2D). Insulin resistance results from compensatory pancreatic β-cell hyperplasia contributing to hyperinsulinaemia that may induce resistance; this phase is followed by eventual β-cell loss [27]. In similar locations to the ZFR *fa* gene are the well characterized *obese* (*ob)* and *diabetes (db)* genes. The product of *ob* is the protein hormone leptin and *db* encodes the leptin receptor in mice (homologous to the *fa* genes in ZFR) [28, 29]. Leptin expressed in adipocytes is thought to serve as a feedback to the brain for fat storage levels and has a functional relation to proinflammatory cytokines [30]. Mutations of leptin (*ob/ob)* or disruption of leptin receptors/signaling pathways (*db/db* mice, *fa/fa* rats) results in obesity syndromes and corresponding T2D models in animals.

Regulation of glucose metabolism by insulin and fatty acids

Glucose utilization by the heart is stimulated by insulin and inhibited by fatty acids, effects attributed to the modulation of limiting steps in glucose metabolism. The distinct metabolic phenotype of the diabetic heart is characterized by elevations in fatty acid uptake and oxidation combined with a decrease in glucose uptake and oxidation, a pattern largely mediated by competition between glucose and fatty acid metabolism as defined by the Randle hypothesis and transcriptional regulation of limiting enzymes. Because the diversion

of metabolites from glycolysis to diabetes-dependent pathological pathways contributes to the development of DCM, these limiting steps become potential sites of pathology [31].

One of the limiting steps in myocardial glucose metabolism is glucose uptake via the glucose transporters GLUT1 and GLUT4. It has been proposed that the basal rate of glucose transport is GLUT1 dependent while the facilitation of glucose transport by insulin involves both the translocation of GLUT4 to the cell membrane and the upregulation of the transporter, an effect that is impaired in diabetes [7]. Both hyperglycemia and hyperlipidemia attenuate insulin-mediated stimulation of glucose transport. However, elevations in plasma glucose enhance glucose uptake through mass action, partially overcoming impaired glucose uptake caused by severe insulin resistance and hyperlipidemia. Thus, despite the dramatic reduction in glucose uptake, the size of the intracellular glucose pool is elevated in the type 1 diabetic heart [32]. Through a similar mechanism, the intracellular glucose pool of the nondiabetic heart is increased by addition of palmitate to the medium [32]. Therefore, impaired GLUT4 activity in the diabetic heart does not limit glycolytic flux, as adequate amounts of glucose are available for the hexokinase reaction. These data imply that key bottlenecks in glycolysis develop downstream from the glucose transporters in the diabetic heart.

One of the most important limiting glycolytic enzymes in the diabetic heart is phosphofructokinase (PFK), which catalyzes the conversion of fructose-6-phosphate to fructose 1,6 bisphosphate. This enzyme is a key target of fatty acid-mediated regulation of glycolysis, as enhanced rates of fatty acid β-oxidation lead to elevations in cardiac levels of acetyl CoA and citrate, the latter a potent inhibitor of PFK [33]. The allosteric enzyme is also a major sensor of the high energy phosphate content of the heart, as both a low ATP/ADP ratio and elevations in AMP kinase activity stimulate the activity of the enzyme [33, 34]. Because diabetes is associated with enhanced rates of fatty acid β- oxidation and elevations in citrate levels, it has been proposed that PFK activity is diminished in the diabetic heart [33]. However, PFK is located in the cytosol, while citrate is largely produced in the mitochondria. Moreover, the observation that tricarboxylate carrier activity in heart mitochondria is low has raised further doubts about the assumption that citrate levels regulate PFK in the diabetic heart [35]. An alternate mechanism for the control of PFK is transcriptional modification. According to Finck et al [36], hearts overexpressing PPARα have reduced PFK expression, although the mechanism of regulation has not been clarified. Some of the most compelling data supporting a role for PFK in diabetes-mediated glycolytic impairment come from crossover analysis of metabolic intermediates of type 1 and lean type 2 diabetic hearts, suggesting elevations in glucose-6-phosphate levels and reductions in fructose 1,6 bisphosphate content [32, 37]. This analysis assumes that substrate levels increase and product levels decrease upon inhibition of the enzyme. Therefore, as a result of reduced PFK activity, a bottleneck develops in the glycolytic pathway of the diabetic heart [32, 37]. The resulting increases in myocardial levels of glucose-6-phosphate and fructose-6-phosphate have pathological consequences, as they serve as substrates for four pathological pathways involved in the development of DCM (Figure 1). Glucose-6- phosphate serves as a substrate for glucose-6-phosphate dehydrogenase, the initial enzyme in the hexose monophosphate shunt. In the obese T2D heart, the generation of NADPH by glucose-6-phosphate dehydrogenase provides substrate for NADPH oxidase, a key enzyme involved in the generation of ROS in the cytosol [38]. Glucose-6-phosphate can also contribute to the glycation of proteins, a reaction capable of generating ROS and damaging key proteins in the diabetic heart [39]. A receptor for advanced glycation end products initiates pathways that appear to contribute to the development of DCM [40]. In a similar manner, the diversion of fructose-6-phosphate to diabetes-linked pathological pathways contributes to the development of DCM. Fructose-6-phosphate initiates the hexosamine pathway, whose final reaction is a transferase-mediated modification of serine or threonine residues leading to the

formation of an O-linked N-acetylglucosamine product. Stimulation of the hexosamine pathway has been linked to impaired calcium cycling and contractile dysfunction of the diabetic heart [41-43]. Glycolytic intermediates have also been implicated in the promotion of the polyol pathway [44]. Although the polyol pathway is reportedly involved in diabetes-mediated vascular injury, a role for the pathway in the development of DCM remains to be established.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is inhibited by high intracellular concentrations of NADH and ATP [33]. Since the levels of GAPDH in the heart are relatively high, the enzyme is not limiting in most pathological conditions. However, during myocardial ischemia, cellular levels of fructose 1,6 bisphosphate and triose-phosphate accumulate without a rise in metabolic intermediates located downstream of the enzyme, suggesting a rate controlling role for GAPDH in glycolysis [33]. Evidence that diabetes leads to respiratory chain dysfunction implies that diabetes must be associated with a rise in the $NADH/NAD^+$ ratio that could inhibit GADPH. However, in the lean type 2 diabetic heart, crossover analysis fails to uncover a bottleneck in glycolysis at the GAPDH site [37] although an increase in glyceraldehyde-3-phosphate levels has been observed [45]. According to Ojaimi et al. [46], GAPDH is downregulated in the heart of type 1 diabetic dogs. Moreover, according to the unifying hypothesis of diabetes proposed by Brownlee and coworkers [31, 47, 48], GAPDH assumes a central role in the development of diabetic complications. In Brownlee's hypothesis, the metabolism of glucose by endothelial cells leads to the formation of ROS in the mitochondria. The oxidants then cross the mitochondrial membrane and migrate to the nucleus where they produce oxidative DNA damage. The oxidized DNA products activate poly(ADP-ribose)polymerase, an enzyme that catalyzes ADP-ribosylation of proteins [49]. GAPDH is taken up by the nucleus, where it undergoes ADP ribosylation, inactivating the enzyme. Du et al. [50] maintain that ADP ribosylation of GAPDH diminishes the levels of active enzyme, forming a bottleneck in glycolysis that allows the diversion of trioses into pathological pathways. Although Brownlee's theory introduced the novel idea that bottlenecks in glycolysis can cause the diversion of glycolytic intermediates into diabetes-linked pathological pathways, questions have been raised regarding the role of GAPDH in the development of one of these bottlenecks [51]. Clearly, further study of the role of GAPDH in the development of DCM is warranted.

Another limiting step of glucose metabolism in the heart is the pyruvate dehydrogenase complex (PDH), which consists of three enzymes. One enzyme catalyzes the irreversible conversion of pyruvate to acetyl CoA and two enzymes regulate the activity of the PDH reaction through phosphorylation/dephosphorylation. Pyruvate dehydrogenase kinase (PDH kinase) catalyzes the phosphorylation and inactivation of the PDH reaction, while pyruvate dehydrogenase phosphatase (PDH phosphatase) catalyzes the dephosphorylation and activation of the complex. The activity of PDH kinase is inhibited by pyruvate and ADP and activated by elevations in the acetyl CoA/CoA and the $NADH/NAD^+$ ratios [33, 52, 53]. PDH phosphatase activity is enhanced by increases in Ca^{2+} and Mg^{2+} [33, 54]. Central to the ability of pyruvate to enter the mitochondria and undergo the irreversible conversion to acetyl CoA is the rate of fatty acid oxidation, which elevates acetyl CoA and inhibits PDH, and the generation of pyruvate by glycolysis, which increases the substrate and activator of PDH. Indeed, the acetyl CoA/CoA ratio and the PDH reaction are highly dependent on the fatty acid, glucose and insulin content of the perfusion medium [55]. The highest acetyl CoA/CoA ratios are observed in hearts perfused with medium containing 1.2 mM palmitate, 20 mM glucose and insulin, while the lowest ratios are detected in hearts perfused with 0.4 mM palmitate and 5 mM glucose in the absence of insulin. Diabetes elevates the acetyl CoA/CoA ratio in hearts perfused with buffer containing high palmitate and glucose content as well as the low substrate, insulin free condition. In accordance with expected PDH

activity, palmitate addition and diabetes severely reduce glucose oxidation while enhancing palmitate oxidation, effects that are not appreciably altered by addition of insulin to the buffer. Severe reductions in glucose oxidation are observed in diabetic hearts perfused with buffer containing low insulin levels and the combination of either high concentrations of palmitate and glucose or low concentrations of the two substrates, indicating that the metabolic effects of diabetes cannot be reversed by acute changes in substrate content and insulin. However, PDH activity can be restored in the hearts of diabetic animals subjected to either chronic refeeding or insulin treatment [33, 52]. Thus, suppression of glucose oxidation in the diabetic heart depends not only upon the acute metabolic interactions that develop between fatty acid and glucose metabolism, but also the metabolic interactions mediated by chronic exposure of the diabetic heart to high circulating levels of fatty acids.

According to Finck et al [36], peroxisome proliferator activated receptor alpha (PPARα) levels are elevated in both T1D and obese T2D hearts, at least in part caused by the increase in fatty acid levels. One of the targets of PPARα is PDH kinase 4, whose expression is upregulated by PPARα [36]. In mice overexpressing PPARα, the upregulation of PDH kinase 4 is associated with reductions in glucose oxidation [55], while the downregulation of PPARα leads to elevated rates of glucose oxidation [56]. Chatham and Forder [57] found that dichloroacetate-mediated stimulation of PDH in the T1D heart significantly increases flux of pyruvate through the citric acid cycle and the overall rate of glucose oxidation, effects that appear responsible for the observed improvement in contractile function. The authors also found that T1D hearts perfused with buffer containing glucose as the sole substrate exhibit significant contractile dysfunction, an effect reversed by the addition of hexanoate to the perfusion buffer. The beneficial effect of hexanoate was attributed to acceleration in the rates of acetyl CoA generation, citric acid cycle flux and reducing equivalent generation.

Inhibition of PDH restricts the entry of pyruvate into the mitochondria while enhancing the accumulation of trioses upstream from pyruvate kinase. The diversion of these trioses into diacylglycerol biosynthesis contributes to the activation of several diacylglycerol-sensitive protein kinase C (PKC) isoforms. Connelly et al [58] have found that inhibition of PKC-β preserves cardiac function in Ren-2 diabetic rats. However, other PKC isoforms may also contribute to the complications of diabetes. Among other adverse actions, PKC activates NADPH oxidase, stimulating the generation of ROS [59].

Regulation of fatty acid metabolism by glucose and insulin

The healthy myocardium preferentially utilizes fatty acids as a source of energy, providing up to 70% of reducing equivalents for ATP synthesis. However, T2D causes a further stimulation in fatty acid uptake and oxidation through an elevation in circulating levels of fatty acid and an upregulation of both fatty acid translocase (FAT/CD36) and fatty acid binding protein (FATP1), proteins involved in fatty acid uptake by the heart [60-62]. Ironically, insulin also promotes both the upregulation of FAT/CD36 and its translocation to the sarcolemma [63, 64]. However, chronic elevations in circulating fatty acids enhance the expression of several enzymes of fatty acid metabolism. These effects are mediated in part by the activation of the PPAR family of nuclear receptors [65], with particular relevance being PPARα, whose activation results in the activation of both FAT/CD36 and FATP1 [62]. In obese T2D rats, the uptake of fatty acids into the heart is associated with enhanced rates of FAT/CD36 translocation to the sarcolemma [60, 62].

The myocardium contains triglyceride stores, which can be rapidly mobilized. Although the size of these stores is normally small, they increase in the diabetic heart. The mobilization of these stores limits the degree of glucose oxidation in glucose-perfused hearts by providing a

source of fatty acids for oxidation. In the obese T2D rat, the accumulation of ceramides contribute to the development of glucose intolerance and is a mediator of apoptosis [66]. Fatty acyl CoA and carnitine accumulation have also been implicated in the development of DCM [67].

One of the most important regulatory steps of fatty acid metabolism is the transport of fatty acids into the mitochondria. Short and medium chain fatty acids are activated in the matrix by acyl CoA synthetase, therefore, their metabolism is independent of carnitine. However, the long chain fatty acids require carnitine to enter the mitochondria, a process catalyzed by carnitine palmitoyltransferase I (CPT-I) and CPT-II. CPT-I, which catalyzes the formation of fatty acyl carnitine from fatty acyl CoA, is allosterically inhibited by malonyl CoA [62]. In type 1 diabetic hearts perfused with medium containing 1.2 mM palmitate, 20 mM glucose and insulin, malonyl CoA levels decline, which mediates an increase in palmitate oxidation [68]. Malonyl CoA synthesis is catalyzed by acetyl CoA carboxylase while the degradation of malonyl CoA to acetyl CoA involves malonyl CoA decarboxylase. Acetyl CoA carboxylase activity is regulated by phosphorylation-dephosphorylation, with AMP kinase activation capable of completely inhibiting the enzyme through phosphorylation [69]. AMP kinase, which is an energy sensor, is stimulated by elevations in the AMP/ATP and creatine/creatine phosphate ratios while being inhibited by insulin, provided that the two ratios remain elevated [70]. The increase in fatty acid oxidation in the T1D and obese T2D heart has been attributed in part to an upregulation in malonyl CoA decarboxylase, an enzyme controlled by high fat feeding, diabetes and elevated PPARα levels [68, 71]. As expected, malonyl CoA decarboxylase inhibitors decrease fatty acid oxidation and enhance glucose oxidation through malonyl CoA-mediated regulation of CPT-1.

The diabetic phenotype does not develop in PPARα null mice [72]. On the other hand, cardiac hypertrophy and fractional shortening are worse in diabetic mice overexpressing PPARα, an effect associated with myocardial accumulation of triglycerides containing long chain fatty acids. Also defective in the diabetic-PPARα overexpressing heart is the upregulation of ACO, an enzyme involved in the generation of ROS in the peroxisomal fatty acid oxidation pathway. It is proposed that ACO may contribute to the observed elevation in ROS generation and decline in the glutathione redox ratio (GSH/GSSG). In a related study, Chen et al [73] showed that treatment of obese T2D mice and PPARα overexpressing mice with Astragalus, an active ingredient of traditional Chinese medicine, improves LV chamber dilatation, fractional shortening dysfunction and the expression of sarcoplasmic reticular Ca^{2+} ATPase 2a (SERCA2a). The contractile effects were correlated with increases in glucose oxidation and decreases in fatty acid metabolism. Recently, decreased PPARδ expression has been implicated in the development of DCM [74]. PPARδ is the predominant member of the PPAR family in the heart, where it regulates glucose and fatty acid metabolism [75]. However, mice overexpressing PPARβ/δ do not develop a cardiomyopathy or exhibit lipid accumulation [76]. Therefore, while the expression of PPARδ is diminished in the diabetic heart, the effects of PPARα appear to be the dominant regulator of glucose and fatty acid metabolism.

Mitochondrial dysfunction in the diabetic heart

The generation of acetyl CoA by fatty acid oxidation and pyruvate dehydrogenase is a key determinant of citric acid cycle flux, with fatty acid oxidation accounting for the increase in acetyl CoA generation by the diabetic heart [77]. Bowman [77] also observed elevations in the levels of citrate, isocitrate, malate and α-ketoglutarate in the T1D heart, changes which were consistent with the stimulation in acetyl CoA synthesis by fatty acid oxidation. Based on similar findings, Taegtmeyer and Passmore [78] suggested that diabetes mediates the unspanning of citric acid cycle flux, as excessive acetyl CoA generation leads to a

deficiency in free CoA, which diminishes flux through α-ketoglutarate dehydrogenase but enhances flux through the transamination of α-ketoglutarate. However, slowing of respiratory chain flux likely contributes to the inhibition of α-ketoglutarate dehydrogenase, as respiratory chain inhibition elevates the $NADH/NAD^+$ ratio, which diminishes citric acid cycle flux [79-82]. Among the respiratory chain complexes depressed in T1D and obese T2D hearts are I, III and V [79-82]. The activity of these complexes is regulated by the levels of mitochondrial encoded proteins, which join with nuclear encoded proteins to form the respiratory chain complexes. The biosynthesis of mitochondria encoded proteins occurs in the mitochondria and depends upon the levels of mitochondrial DNA, which are determined by the transcriptional activity of mitochondrial transcription factor A (tfam). Suarez et al [83] discovered that overexpression of tfam dramatically affects the function of neonatal rat cardiomyocytes incubated in medium containing 30 mM glucose. While control cells exposed to high glucose exhibit a delay in the relaxation phase of the Ca^{2+} transient, a reduction in cellular ATP content and diminished SERCA2a content, cells containing elevated levels of tfam show an improvement in Ca^{2+} delay and elevations in both cellular ATP and SERCA2a content. Hyperglycemia also increases the levels of O-GlcNAcylated tfam, which diminish the activity of tfam and reduce the activity of oxidative phosphorylation (complex V activity). Interestingly, chronic exposure of nondiabetic rats to the PPARα agonist Wy-14,643 leads to reductions in state 3 respiration, but elevations in state 4 respiration of the heart, indicating that the rate of ADP phosphorylation is significantly depressed in Wy-14,643 treated hearts [84]. The depression in respiration in Wy-14,643 treated rats was attributed to reductions in cyclooxygenase II (COXII), a mitochondria encoded protein subunit of cytochrome oxidase. Another consequence of impaired respiratory chain flux is the generation of ROS as electrons are diverted from the respiratory chain to the acceptor oxygen. Boudina et al [85] reported that heart mitochondria from obese T2D mice produce elevated rates of ROS. Besides causing mitochondrial damage, matrix ROS generation can affect cytosolic ROS formation. As shown in Figure 1, ROS from the mitochondria can leave the mitochondria as H_2O_2 and activate PKC in the cytosol. ROS-mediated PKC activation can significantly increase cytosolic ROS content by activating NADPH oxidase. Thus, diabetes-mediated mitochondrial dysfunction contributes to the development of DCM by altering ATP generation and Ca^{2+} movement.

Role of impaired energy metabolism in the development of DCM

Brownlee [31] introduced the novel hypothesis that the complications of diabetes arise from the development of a bottleneck in glycolysis that diverts glycolytic intermediates into diabetes-linked pathological pathways (Figure 1). The theory was based on the observation that glucose-treated bovine aortic endothelial cells generate superoxide that plays a central role in the development of diabetic complications [48]. Inhibition of mitochondrial superoxide generation prevents the activation of several pathological pathways of hyperglycemic injury (protein kinase C stimulation, hexosamine pathway, polyol pathway and glycation end product formation). In a follow-up study, Du et al. [50] localized the glycolytic bottleneck to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with more recent studies attributing the decline in GAPDH activity to ADP-ribosylation, a reaction mediated by poly (ADP-ribose)polymerase [49]. It was proposed that oxidants generated in the mitochondria enter the nucleus, causing oxidative DNA damage that stimulates poly (ADP-ribose) polymerase activity. Schaffer et al. [51] have questioned the role of ADP-ribosylation in the regulation of glycolysis in diabetes, as both GAPDH uptake by the nucleus and its ADP-ribosylation are commonly associated with cell death. Since diabetes leads to respiratory chain dysfunction, a more likely cause of GAPDH inhibition is the rise in the $NADH/NAD^+$ ratio.

Treatment Strategies for DCM

Glycemic Control

Hyperglycemia has been shown to be instrumental in the development of DCM in T2D patients. Consequently, with improved glycemic control, diastolic dysfunction is also improved [86, 87]. Thiazolidinediones (TZDs) activate PPAR-γ allowing for increased peripheral insulin sensitivity and secondarily reduce hepatic glucose production, reducing hyperglycemia. Although effective glycemic controllers that decrease the effect of DCM, these drugs are contraindicated in advanced cases of heart disease (New York Heart Association classes III and IV). Thiazolidinediones also improve adipocyte function and can alter adipocyte storage and triglyceride levels [88, 89]. Metformin, a biguanide class antidiabetic drug, operates via decreasing hepatic glucose production and has demonstrated improvements in survival of T2D patients with heart failure and can also decrease the prevalence of heart failure development in T2D patients. Studies do not offer direct information on the effects of metformin on DCM, but the reduction in CVD rates justifies its usage to treat DCM [90, 91]. New agents utilizing incretin for glycemic control appear to have beneficial effects and reduce heart failure in DCM. Incretin mimetics include injectable glucagon like peptide-1 (GLP-1) analogs that activate the GLP-1 receptors, thereby increasing glucose dependent insulin secretion and decreasing glucagon secretion. Likewise, oral dipeptidyl peptidase inhibitors prolong the half-life of endogenous GLP-1, increasing its effect. Overall, these agents have a positive effect on myocardial function and left ventricular ejection fraction in patients with heart failure in both clinical studies and animal models [90, 92, 93]. Alpha-glucosidase inhibitors, specifically acarbose, prevent the absorption of glucose in the gut: increasing the effect of insulin, decreasing stress on β-cells and reducing CVD risk due to postprandial hyperglycemia [94, 95]. Additionally, for type I DM, it has been shown that DCM does not develop if the T1D is well controlled with insulin therapy. These data point to hyperglycemia not only as an exacerbating, but also a treatable factor of DCM [96].

β-Adrenergic Blockade

Significant evidence has accumulated showing the benefits of β-blocker therapy in treating heart failure and left ventricular systolic dysfunction. Meta-analysis of multiple trials with focus on DM patients confirms the benefits of β-blockade in heart failure patients with diabetes. Also shown is that β-blockers with an additional treatment such as angiotensin converting enzyme inhibitors (ACEIs) show increased relative risk reduction in mortality rates of diabetic congestive heart failure (CHF) patients [97, 98].

Cholesterol Reduction

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins, HMG-CoA reductase inhibitors) reduce the synthesis of cholesterol and have a multitude of effects known to reduce the incidence of DCM. Statins have also been shown to reduce mortality in patients with ischemic and non-ischemic heart failure, however some disagreement exists with the results [99]. In the realm of DCM, not enough studies have been performed to allow definitive indications for the effective use of statins.

Exercise Training

Exercise training has been shown to prevent and improve DCM. Reviews of system studies show that exercise training reverses DM induced free fatty acid metabolism, increases sensitivity to insulin, allows for increased glucose metabolism, increases cardiac output and can even reverse contractile dysfunction observed in DCM [18]. A study in T2D mice found that interval training reverses myocyte Ca^{2+} handling dysfunction. Mice that underwent the

training protocol exhibited a return to normal sarcoplasmic reticulum Ca^{2+} load and a decrease in spontaneous Ca^{2+} waves. The training did not lead to normal blood glucose or insulin levels, suggesting that it may have had a more direct effect on the heart [100]. In recent studies of vigorous long lasting exercise in a T1D model (STZ-induced), results demonstrated altered dynamics of cardiomyocyte contraction, however they did not show a change in amplitude of contraction or calcium transients, implicating a possible effect of exercise on other states of Ca^{2+} homeostasis [101]. These results illustrate positive overall effects of exercise training in combating DCM.

Fibrates

Fibrates are a class of drugs acting as PPARα agonists with the demonstrated ability to lower triglycerides/low density lipoproteins (LDL) levels, raise high density lipoproteins (HDL) levels and increase insulin sensitivity in humans and animals [102, 103]. These actions make fibrates an attractive treatment option for the hyperlipidemia and hyperinsulinemia that accompany T2D. Additionally, clinical trials have shown that fibrate therapy may reduce CVD risk factors associated with dyslipidemia and decrease occurrence of coronary events in the diabetic populations [104]. More specifically for diabetic cardiomyopathy, fibrate treatment (bezafibrate) has been shown to prevent not only metabolic dysfunction in sucrose fed animals, but also prevent cardiomyocyte dysfunction seen in these T2D models [105]. The clinical effects of fibrates and other classes of PPARα agonists should continue to be pursued to determine possible protective effects against type 2 DCM.

Antioxidants

Antioxidants may also be a promising preventative treatment strategy for DCM. A study in pre-diabetic insulin resistant mice showed a significant increase in ROS production, along with abnormal Ca^{2+} signaling and decreased Na^{+}/Ca^{2+} exchanger protein expression. Transgenic mice with an overexpression of catalase, an enzyme that catalyzes the breakdown of hydrogen peroxide, restored normal Ca^{2+} signaling and contractile properties despite having no effect on insulin sensitivity and post-insulin receptor signaling [106]. As mentioned above, metallothionien overexpression relieved almost all of the cellular DCM dysfunction except for decreased SERCA2a function, however contractile parameters were restored [107]. This suggests that antioxidants may be an important treatment for DCM.

RAAS Modification

Hyperglycemia has been shown to trigger the renin angiotensin aldosterone system (RAAS), which is known to directly affect the heart. Angiotensin II depresses Ca^{2+} sparks and inhibits SERCA2a in heart failure. Candesartan, an angiotensin II type 1 receptor blocker (ARB), has been shown to restore normal sparks in atrial tissue that are impaired by Angiotensin II. Based on this, candesartan was administed to myocytes isolated from STZ-induced diabetic rats, which reduced PKC levels and restored Ca^{2+} transients and sparks and SR Ca^{2+} loading [108]. These results indicate that further research on candesartan and angiotensin II receptor blockers at the *in vivo* level may show improvements in Ca^{2+} signaling parameters in DCM. Angiotensin convering enzyme inhibitors (ACEIs) block the conversion of angiotensin I to angiotensin II, resulting in increased levels of bradykinin. This increased vasoactivity can facilitate blood flow to muscle and adipose tissue including cardiac tissue, leading to reduced effects on cardiovascular fibrosis. ACEIs have been shown to decrease CVD rates and all-cause mortality in patients with diabetes, in particular diabetic hypertensive patients. Also, treatment with ACEIs has been shown to increase insulin sensitivity of cells [109-111]. Angiotensin II receptor blockers (ARBs) and ACEI can prevent coronary perivascular fibrosis and collagen deposition [112]. Along with the

aforementioned medications, aldosterone antagonists have been shown to cause positive remodeling in patients with LVDD, an effect that may be applicable to T2D [113]. These drugs modifying the RAAS system have significant positive effects for the treatment of DCM.

Calcium Upregulation

Calcium channel blockers (CCBs) can increase intracellular calcium to prevent DM-induced cardiomyocyte changes. Diltiazem showed possible suppression of muscle hypertrophy, degeneration and fibrosis caused by hyperglycemia in animal T1D models [114]. Early studies with verapamil demonstrated marked improvement of myosin/myofibrillary ATPase and SERCA activity in ventricular tissue of STZ induced T1D animals. Additionally, decreases in diastolic ventricular pressure and increases in rate of contraction and relaxation were all shown in STZ-treated mice [115, 116]. These data suggest the use of verapamil in treating type 1 DCM. STZ treated animals similarly saw a beneficial effect in insulin sensitivity and cardiac function, as well as reductions in hypertriglyceridemia from treatments with nifedipine [117, 118]. Clearly, there is a potential for use for CCBs in DCM, however additional studies are required to determine the mechanisms of action of CCBs in DCM.

Future Treatment Strategies

Recent years have yielded new therapies for DCM focusing on advanced glycation endproducts (AGEs) including AGE inhibitors and AGE cross-link breakers, which may provide future directions for DCM intervention, treatment and prevention [119].

The calcium signaling pathway lends itself to several potential DCM treatment targets. The most obvious therapeutic strategy for impaired calcium signaling is improving SERCA2a function. Overexpression of SERCA2a in transgenic rats prevented drug-induced DM-induced contractile dysfunction by inhibiting changes in contractile function and calcium handling. A follow up study showed that doxycycline-induced SERCA2a overexpression following the induction of DCM improved contractile and calcium signaling parameters [120]. This both confirms the importance of SERCA2a downregulation in DCM and suggests a potential therapeutic target for patients. Overall, PKC inhibition in preventing DCM via the calcium signaling pathway seems to be worth pursuing as PKC appears to prevent changes in expression of calcium handling proteins [121].

There are numerous options for the treatment of DCM (Table 1), but the best treatment option will depend on, among other things, the type of DM, the individual’s response to prescribed therapies and complicating comorbidities.

Conclusions

The epidemic rise in DM worldwide has made DCM an increasing health concern. In the present review, we introduce a modified version of the Brownlee hypothesis. We suggest that inhibition of phosphofructokinase (PFK) leads to the formation of a bottleneck that diverts hexose intermediates into the hexosamine pathway, glycosylated end products, the polyol pathway and the hexose monophosphate shunt (Figure 1). The role of these pathways in the generation of ROS, protein damage and calcium mishandling are widely recognized. PFK is inhibited by citrate, which increases as a result of enhanced fatty acid metabolism. Also regulating PFK is fatty acid-mediated elevation in PPARα. The other major bottleneck in the glucose oxidation pathway is pyruvate dehydrogenase (PDH), whose inhibition by phosphorylation is mediated by the fatty acid-mediated accumulation of acetyl CoA and PPARα. Inhibition of PDH leads to the accumulation of trioses that are diverted into

diacylglycerol, an activator of protein kinase C. Several PKC isoforms (α, β, δ ,ε and ξ) are activated in the diabetic heart and glucose-treated cardiomyocytes [122- 126]. Overexpression of PKC-β_2, whose levels are upregulated in diabetes, leads to the development of a cardiomyopathy [127]. Moreover, PKCδ plays a role in apoptosis, which is enhanced in diabetes [125]. The activation of PKCε is associated with the phosphorylation of troponin I and troponin T, which inhibits myofibrillar ATPase [124]. PKC has also been implicated in the generation of ROS by NADPH oxidase. Thus, the regulation of PDH represents an important bottleneck site for the modulation of contractile function. Additionally, we offer a variety of treatment options shown to be effective in treating DCM (Table 1) including glycemic control, adrenergic blockade, calcium up-regulation, RAAS modification, antioxidant treatment and exercise training.

ABBREVIATIONS

ACEI	angiotensin converting enzyme inhibitor
ADP	adenosine diphosphate
AGES	advanced glycation end products
AMP	adenosine monophosphate
ARB	angiotensin receptor blockers
ATP	adenosine triphosphate
BMI	body mass index
CASQ2	calsequestrin
CCB	calcium channel blockers
CHF	congestive heart failure
COX	cytochrome oxidase
CPT-1	carnitine palmitoyltransferase-1
CVD	cardiovascular disease
DCM	diabetic cardiomyopathy
DM	diabetes mellitus
FAT	fatty acid translocase
FKBP	FK 506 Binding Protein
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter
GSH/GSSG	glutathione redox ratio
HMG-COA	3-hydroxy-3-methylglutaryl coenzyme A
LV	left ventricle
LVDD	left ventricular diastolic dysfunction
LVH	left ventricular hypertrophy
NADPH	nicotinamide adenine dinucleotide phosphate
NCX	Na^+/Ca^{2+} exchanger

O-GlcNAC	O-linked N-acetylglucosamine
PDH	pyruvate dehydrogenase complex
PFK	phosphofructokinase
PKC	protein kinase C
PPAR	peroxisome proliferation activated receptor
RAAS	renin angiotensin aldosterone system
ROS	reactive oxygen species
SERCA2A	sarco(endo)plasmic reticulum calcium ATPase
SR	sarcoplasmic reticulum
STZ	streptozotocin
TFAM	mitochondrial transcription factor A
TZD	Thiazolidinediones
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
ZFR	Zucker fatty rats

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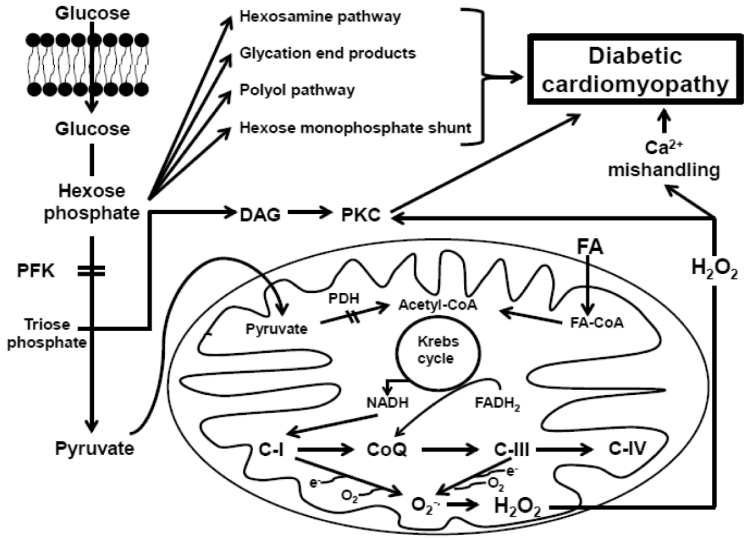

Figure 1.

Metabolic Mechanisms of Diabetic Cardiomyopathy PFK- phosphofructokinase; DAG – diacylglycerol; PKC – protein kinase C; FA – fatty acid; PDH – pyruvate dehydrogenase; NADH – nicotinamide adenine dinucleotide (reduced); FADH – flavin adenine dinucleotide (reduced); CoQ – coenzyme Q; CI, III, IV – respiratory chain complexes

Table 1

Therapeutic Strategies for Diabetic Cardiomyopathy

Diabetic Cardiomyopathy Treatment	Therapeutic	strategy DCM Type
Glycemic Control:	Insulin therapy	T1D
	Thiazolidinediones	T2D
	Metformin	T2D
	Glucagon-like peptide-1 Dipeptidyl peptidase inhibitors	T2D
	Alpha-glucosidase inhibitors	T2D
Adrenergic Blockade:	β-blockers	T1D & T2D
Cholesterol Reduction:	Statins	Efficacy Questionable for DCM
Exercise Training:	–	T1D & T2D
Antioxidants:	–	T1D & T2D
RAAS:	ACEIs	T1D & T2D
	ARBs	T1D & T2D
	Aldosterone Antagonist	T1D & T2D
Calcium Upregulation:	Calcium Channel Blockers	Efficacy Questionable for T1D

ACEI – Angiotensin converting enzyme inhibitor, ARB- Angiotensin receptor blockers, T1D – type 1 diabetes mellitus, T2D – type 2 diabetes mellitus.