The vulnerable myocardium
Diabetic cardiomyopathy

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Summary
Cardiovascular disease is the major cause of morbidity and mortality in subjects suffering from diabetes mellitus. While coronary artery disease is the leading cause of cardiac complications in diabetics, it is widely recognized that diabetes increases the risk for the development of heart failure independently of coronary heart disease and hypertension. This increased susceptibility of the diabetic heart to develop structural and functional impairment is termed diabetic cardiomyopathy. The number of different mechanisms proposed to contribute to diabetic cardiomyopathy is steadily increasing and underlines the complexity of this cardiac entity.

In this review the mechanisms that account for the increased myocardial vulnerability in diabetic cardiomyopathy are discussed.

Diabetes mellitus increases the risk of developing cardiovascular disease (1) because it

• accelerates the development of coronary atherosclerosis,

• increases the risk for myocardial infarction, and

• impairs the clinical outcome following acute cardiovascular events (2).

While coronary heart disease is the major cause of cardiac complications in diabetics, it is widely recognized by now that the risk for the development of heart failure remains increased in the absence of coronary artery disease and hypertension. Several decades ago, Rubler and colleagues reported of four diabetic patients that died from heart failure without common causes of heart failure, including hypertension, ischaemic heart disease, or valvular or congenital heart disease (3). This observation was confirmed by several subsequent studies by other groups, reporting an increased heart failure risk in diabetic patients independent of myocardial infarction and hypertension, or reporting an increased heart failure risk in diabetic patients even after adjusting for age, blood pressure, body weight, cholesterol and coronary artery disease (4, 5).

Thus, the term diabetic cardiomyopathy was coined, defined as ventricular dysfunction in diabetic individuals in absence of coronary heart disease and hypertension.

Based on numerous observations, including data reported from the Strong Heart Study, the Cardiovascular Health Study and numerous rodent model studies, cardiac hypertrophy and diastolic dysfunction are believed to be typical manifestations of diabetic cardiomyopathy (4, 6). Systolic dysfunction is usually not observed by conventional echocardiography, although subtle impairment in systolic function has been described in 24% of diabetic patients without coronary heart disease and hypertension using strain analysis and peak systolic velocity measurements (7). If a strict definition of structural changes leading to clinically relevant cardiac dysfunction is applied, some people might argue that a distinct cardiomyopathy of diabetes may not exist. However, many structural and molecular alterations have been reported both in animal models and humans that would predict adverse cardiac outcome following additional stressors, and that overlap with mechanisms proposed to contribute to the development of heart failure. Thus, diabetic cardiomyopathy may rather be interpreted as a predisposition to develop cardiac dysfunction in the presence of additional stressors. The current review provides an overview of molecular mechanisms that have been proposed to underlie the development of diabetic cardiomyopathy (Fig. 1).
Diabetic Cardiomyopathy

Molecular Mechanisms:
- AGE
- Increased Fibrosis
- Increased Cell Death
- Increased Inflammation
- Increased RAAS Activation
- Impaired Ca\(^{2+}\)-Handling
- Increased FA Utilization
- Lipotoxicity
- Mitochondrial Dysfunction
- Oxidative Stress
- Endoplasmic Reticulum Stress
- Micro-RNAs

Clinical Features:
- Cardiac Hypertrophy
- Diastolic Dysfunction

Heart Failure Risk

Fig. Potential mechanisms and clinical features of diabetic cardiomyopathy, resulting in increased risk for the development of heart failure (AGE: advanced glycation endproducts; RAAS: renin-angiotensin-aldosterone system; FA: fatty acid)

Mechanisms of diabetic cardiomyopathy

Advanced glycation end products

Advanced glycation end products (AGEs) are mainly glycosylated proteins as a consequence of non-enzymatic binding of free reducing sugars and reactive carbonyls to proteins. AGE accumulate in a hyperglycaemic environment, and the modification may result in altered protein function and/or maladaptive adaptations. For example, AGE can cause crosslinks in collagen molecules, thereby increasing myocardial fibrosis (8). The sarcoplasmic reticulum Ca\(^{2+}\)-ATPase 2a (SERCA2a) is also modified by AGE, which may impair sarcoplasmic reticulum Ca\(^{2+}\) reuptake in cardiac myocytes (9, 10). AGEs may also bind to a number of receptors, including the AGE receptor (RAGE). Increased activation of RAGE in diabetes may activate various intracellular processes, including expression of inflammatory cytokines (e.g. via MAP kinase signaling, NF\(\kappa\)B signaling, and increased reactive oxygen species production), cell proliferation (via JAK-STAT signaling), profibrotic signaling, apoptosis (via p53 and calcineurin signaling) and autophagy (11, 12). Prevention of dp/dt and left ventricular developed pressure in streptozotocin (STZ)-diabetic rats with knockdown of RAGE emphasizes the functional relevance of the AGE-RAGE axis in the pathophysiology of diabetic cardiomyopathy (13). Thus, AGE and the AGE-RAGE axis may contribute to both structural and functional alterations in diabetic cardiomyopathy.

Increased fibrosis

Both perivascular and intermyofibrillar fibrosis have been shown to be increased in human myocardial samples of diabetic patients without evidence of coronary artery disease and hypertension (14, 15). Myocardial connective tissue content is also increased in rodent models of Type 1 and Type 2 diabetes (16, 17). Increased expression of the transcription factors transforming growth factor \(\beta\) (TGF\(\beta\)) and connective tissue growth factor (CTGF), or increased activation of poly(ADP-ribose)polymerase-1 (PARP-1) may drive increased collagen deposition in diabetic hearts (18). In addition, reduced extracellular matrix degradation due to lower levels of matrix metalloproteinase-2 (MMP-2) may contribute to increased connective tissue content (19).

Increased inflammation

Proinflammatory mechanisms contribute to the pathogenesis of diabetes (20), and several groups reported increased myocardial expression of cell adhesion molecules, increased infiltration of macrophages and leukocytes, and increased levels of proinflammatory cytokines (21–23). Various interventions reducing myocardial inflammation also attenuate disadvantageous molecular alterations or improve cardiac function in diabetic cardiomyopathy, including angiotensin-1 (AT-1) receptor antagonism, activation of the kallikrein-kinin system, inhibition of p38 MAP kinase signalling, inhibition of interleukin converting enzyme, anti-tumour necrosis factor a (TNF\(\alpha\)) treatment, and inactivation of glycogen synthase kinase 3\(\beta\) (GSK-3\(\beta\)) (21, 22, 24–26).

Increased cell death

Increased cell death is frequently observed both in human and rodent hearts in Type 1 and Type 2 diabetes, including both apoptotic and necrotic cell death (27–31). Increased rates of apoptosis in diabetic hearts may result from increased reactive oxygen species (ROS) production, increased circulating inflammatory cytokines and chemokines, endoplasmic reticulum (ER) stress, activation of the TGF\(\beta\) signaling pathway, increased activation of the local renin-angiotensin-aldosterone system (RAAS), or insulin-like growth factor 1 (IGF-1) resistance (31–34). Increased necrosis may result from increased PARP-1 activation, increased angiotensin II levels, or impaired IGF-1 signalling (32).

Increased intrinsic RAAS activation

The myocardial RAAS is activated in the diabetic heart, and antagonist treatment attenuates cardiac fibrosis in diabetic mice (16, 22). Cardiac angiotensin II receptor density and synthesis are also increased in Type 1 diabetic rodent hearts, and treatment with angiotensin converting enzyme inhibitors partially inhibits increased myocardial superoxide production and apoptosis (16, 35).

Impaired Ca\(^{2+}\) handling

Excitation of the cardiomyocyte results in increased Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels, which in turn triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum, resulting in actin myosin interaction and mechanical work. Relaxation occurs when Ca\(^{2+}\) is actively reimported into the SR by SERCA2a. Myocardial Ca\(^{2+}\) handling is impaired on many levels in Type 2 diabetic hearts, including elevated intracellular resting Ca\(^{2+}\) concentrations, prolonged intra-
cellular Ca$^{2+}$ decay, slowing of Ca$^{2+}$ transients, decreased SERCA2a activity, impaired SR Ca$^{2+}$ reuptake, Ca$^{2+}$ leakage from the SR, and decreased SR Ca$^{2+}$ load (36–38). Similarly, intracellular Ca$^{2+}$ handling is also impaired in Type 1 diabetic rodent models, including attenuated SR Ca$^{2+}$ release and reuptake, delayed recovery of the intracellular Ca$^{2+}$ transient, reduced expression of SERCA2a, and impaired mitochondrial Ca$^{2+}$ handling (39, 40).

**Increased fatty acid utilization**

Studies both in the human and rodent heart have demonstrated that myocardial fatty acid uptake and oxidation are increased, both due to increased fatty acid delivery to the heart and due to increased activity of peroxisome proliferator-activated receptor α (PPARα), which increases fatty acid oxidative capacity by increasing fatty acid oxidation gene expression (41–43). A concomitant decrease in glucose uptake, glycolysis and glucose oxidation results from decreased glucose transporter 4 (GLUT4)-mediated glucose uptake and impaired pyruvate dehydrogenase activity (44–46). The increase in fatty acid utilization is accompanied by increased myocardial oxygen consumption, which is not accompanied by an equivalent increase in cardiac work, thus resulting in decreased cardiac efficiency (cardiac work per oxygen consumed) (42, 47). The increase in oxygen consumption and the decrease in cardiac efficiency likely result from mitochondrial uncoupling, induced by increased fatty acid utilization, which increases mitochondrial ROS that directly activate uncoupling protein activity (48). Mitochondrial uncoupling leads to decreased ATP regeneration and an inadequate increase in cardiac work, but also to increased (uncoupled) oxygen consumption (49). Surprisingly, fatty acid-induced ROS-mediated mitochondrial uncoupling does not occur in certain models of Type 1 diabetes, suggesting partially distinct mechanisms underlying changes in myocardial energy metabolism in the different types of diabetes (41). Finally, mice with cardiomyocyte-restricted knockout of the insulin receptor (CIRCO mice), as a model of cardiomyocyte insulin resistance, also exhibit ROS-mediated mitochondrial uncoupling and a fatty acid-induced impairment in cardiac efficiency, suggesting that the cardiac metabolic insulin resistance observed in some models of type 2 diabetes may contribute to impaired mitochondrial coupling and cardiac efficiency following onset of diabetes (50, 51).

**Lipotoxicity**

Myocardial lipid accumulation occurs in most animal models of diabetes to varying extents, and also in human diabetic hearts (52, 53). Harmful lipids include free fatty acids, acyl-CoAs, acyl-carnitines, diacetyl-glycerols, ceramides and oxidized phospholipids, whereas myocardial triglyceride accumulation may protect from toxic effects of reactive lipid intermediates (54, 55). Mechanisms of lipotoxicity may include increased rates of apoptosis, increased generation of reactive oxygen and nitrogen species, activation of proinflammatory pathways, remodelling of the mitochondrial membrane phospholipid composition, ER stress, and defective insulin signalling (56–60).

**Mitochondrial dysfunction**

Mitochondrial dysfunction is thought to contribute to the development of diabetes and its complications, including diabetic cardiomyopathy. Numerous studies have been published reporting an impairment in mitochondrial oxygen consumption rates, increased mitochondrial oxidative stress, and alterations in mitochondrial ultrastructure in hearts of models of type 1 and type 2 diabetes (52). Recently, these findings have been confirmed in the human diabetic heart as well (61). The underlying mechanism of impaired mitochondrial function in diabetic hearts includes alterations in oxidative phosphorylation (OXPHOS) subunit expression, oxidative damage of proteins and DNA, impaired mitochondrial calcium handling, and impaired cardiac insulin signalling (49, 62).

**Oxidative stress**

Oxidative stress is a well-characterized contributor to complications in diabetes, including cardiac complications. ROS damage proteins, phospholipids, and mitochondrial and nuclear DNA, oxidize lipids to harmful lipid peroxides, and increase the generation of reactive nitrogen species. Evidence of increased mitochondrial H$_2$O$_2$ and lipid peroxide generation, as well as ROS-induced mitochondrial protein modifications have been reported both in type 2 diabetic human hearts and both type 1 and type 2 diabetic rodent hearts (48, 61, 63, 64). More mechanistically, work from Epstein’s group demonstrated at least partial restoration of mitochondrial function and cardiomyocyte contractility in type 1 diabetic OVE26 mice with overexpression of the mitochondrial antioxidants catalase or manganese superoxide dismutase (65, 66). In addition, NADPH oxidase-derived ROS production is increased in hearts of type 1 and type 2 diabetes models, suggesting that oxidative stress in diabetic hearts results from both mitochondrial and extramitochondrial sources (67, 68).

**Endoplasmic reticulum stress**

Endoplasmic reticulum (ER) stress describes the accumulation of unfolded proteins due to a disturbance of protein folding and modification in the ER, ultimately ending in apoptotic cell death. ER stress has been identified to contribute to myocardial apoptosis in animal models of type 1 and 2 diabetes, as evidenced by upregulation of signalling proteins of the unfolded protein response and ER stress-related apoptotic signalling proteins (69, 70). ER stress itself may be mediated by increased oxidative stress in diabetic cardiomyopathy (71).

**Micro-RNAs**

Micro-RNAs are short endogenous, non-coding, single-strand RNAs which decrease target gene expression by direct degradation of target mRNA or by repressing translation of target mRNA. Recent publications support a role for several micro-RNAs in the regulation of systemic glucose metabolism and insulin sensitivity, thus also implicating micro-RNAs in the pathogenesis of type 2 diabetes and related cardiovascular complications (72, 73). Re-
Regarding the diabetic heart, a study in type 1 diabetic Akita mouse hearts revealed several differentially regulated micro-RNAs, suggesting a relevance for micro-RNAs in the development of diabetic cardiomyopathy (74). Studies demonstrated that downregulation of myocardial miR-133a levels may increase myocardial fibrosis in STZ-induced diabetes, and that increased expression of miR-143 may suppress insulin-stimulated glucose uptake in the diabetic heart (75, 76). Downregulation of miR-373 has also been proposed to induce cardiac fibrosis in the diabetic heart via p300 (77). The rapidly growing area of micro-RNA research promises to elucidate more roles of micro-RNAs in the development of diabetic cardiomyopathy in the near future.

**Novel, underinvestigated mechanisms of diabetic cardiomyopathy**

Some novel and to date less investigated mechanisms have been proposed to contribute to diabetic cardiomyopathy. Autophagy is a physiologic process by which proteins, ribosomes, lipids and entire organelles are engulfed by double-membrane structures which are subsequently targeted to lysosomes for degradation (78). Alterations in autophagy have been implicated in myocardial ischaemia–reperfusion injury, cardiac hypertrophy and heart failure (79, 80). In type 1 diabetic mice (streptozotocin model or OVE26 mice) cardiac dysfunction was associated with repression of myocardial autophagy (81, 82). It remains however unclear whether autophagy repression is adaptive or maladaptive in diabetic cardiomyopathy. Restoration of AMP-activated protein kinase (AMPK) activity by metformin treatment increased autophagic activity and improved cardiac function in diabetic hearts, suggesting that decreased autophagic activity may actually contribute to cardiac dysfunction in these models (81). In contrast, diabetic cardiac injury was not ameliorated but further exacerbated in the presence of beclin-1 overexpression in another study, supporting the idea that suppression of autophagy may be adaptive (82). Interestingly, in the same study, the removal of damaged mitochondria by mitochondria-targeted autophagy, also termed mitophagy, appeared to be maintained. This observation prompted the authors to speculate that diabetes may inhibit autophagy and concurrently maintain normal levels of mitophagy to promote removal of dysfunctional mitochondria and thereby limit diabetic cardiac injury. More studies are required to understand the role of autophagy in diabetic cardiomyopathy.

Epigenetics describes inheritable changes in gene expression patterns that are not caused by alterations of DNA sequence. Dysregulation of histone acetylation is a major epigenetic regulatory mechanism of gene expression that contributes to the development of a variety of diseases, and inhibition of histone deacetylases is discussed as therapeutic option to potentially treat a number of prevalent disorders, including cardiovascular diseases (83). In hearts of type 2 diabetic db/db mice, renal failure induced by unilateral nephrectomy increased myocardial acetylation of histone 3 at lysine 23 and 9, which correlated with increased expression of cardiomyopathy-related genes and cardiac hypertrophy, suggesting an interaction between uremia and cardiac hypertrophy in type 2 diabetes via a mechanism mediated by epigenetic modifications of histone H3 in cardiomyocytes (84). DNA methylation of nuclear genes represents another important epigenetic mechanism. Both demethylation and hypermethylation have been shown to regulate gene expression in cardiomyocytes of STZ-diabetic rats (85). While epigenetics is an underinvestigated mechanism in diabetic cardiomyopathy to date, the potential to regulate expression of the entire genome suggests as yet unidentified contributions to the pathogenesis of diabetic cardiomyopathy.

**Limitations, areas of improvement**

Our understanding of the underlying mechanisms of diabetic cardiomyopathy is limited by the use and also limitations of various different models of type 1 and type 2 diabetes (52). It needs to be emphasized that certain molecular alterations are different between type 1 and type 2 diabetic mouse models. For example, impaired cardiac efficiency, mitochondrial uncoupling and mitochondrial oxidative stress are present in hearts of type 2 diabetic db/db mice but not in type 1 diabetic Akita mice (41, 48).

Next, there is no perfect model for each type of diabetes. For example, data generated in mouse models of diabetes in which diabetes results from impaired leptin action (i.e. ob/ob mice, db/db mice) may be confounded by leptin-specific effects, including alterations in myocardial energy metabolism (86). Most data on the effect of type 1 diabetes and diabetes at all in rodents have been generated in the streptozotocin model, but this drug exerts extrapancreatic toxic effects, such as p38 MAP kinase-dependent myocardial oxidative stress (87, 88).

Furthermore, most models are investigated on a pure genetic background, which does not reflect the human genetic heterogeneity and therefore does not easily allow to conclude that the findings in animal models have similar relevance in the human heart. In addition, there is only few studies published to date that tried to confirm findings in animal studies in the human heart, likely due to the difficulty in generating appropriate and sufficient amounts of human tissue. Thus, future areas of improvement may include identification of novel models of type 1 and type 2 diabetes that more closely resemble the pathogenesis of diabetes in humans, and to encourage studies in humans to confirm the relevance of findings in animal studies for human disease. The latter aspect is particularly important since prospective longitudinal studies proving the true pathologic consequences of diabetic cardiomyopathy in humans are still lacking.

**Conclusions**

Diabetic cardiomyopathy is increasingly recognized as a distinct cardiac entity which will increase in parallel with the obesity epidemic. As discussed, numerous alterations have been identified thus far which may causally contribute to the development of diabetic cardiomyopathy.
This vulnerable myocardium increases the likelihood of developing cardiovascular complications and heart failure.

Thus, novel therapeutic approaches need to be developed, which requires further understanding of the basic mechanisms underlying diabetic cardiomyopathy. Some interventions have demonstrated beneficial effects on the associated pathologic features of diabetic cardiomyopathy in preclinical models, and the most promising approaches should be translated from preclinical models to humans.

Conflict of interest

The authors declare that they have no conflict of interest.

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