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Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy

Nikole J. Byrne[a], **Namakkal S. Rajasekaran**[b,c,d], **E Dale Abel**[e], **Heiko Bugger**[a,*]

[a]Division of Cardiology, Medical University of Graz, Graz, Austria

[b]Cardiac Aging & Redox Signaling Laboratory, Molecular and Cellular Pathology, Department of Pathology, Birmingham, AL, USA

[c]Division of Cardiovascular Medicine, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA

[d]Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, USA

[e]Fraternal Order of Eagles Diabetes Research Center, Division of Endocrinology and Metabolism, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, USA

Abstract

Even in the absence of coronary artery disease and hypertension, diabetes mellitus (DM) may increase the risk for heart failure development. This risk evolves from functional and structural alterations induced by diabetes in the heart, a cardiac entity termed diabetic cardiomyopathy (DbCM). Oxidative stress, defined as the imbalance of reactive oxygen species (ROS) has been increasingly proposed to contribute to the development of DbCM. There are several sources of ROS production including the mitochondria, NAD(P)H oxidase, xanthine oxidase, and uncoupled nitric oxide synthase. Overproduction of ROS in DbCM is thought to be counterbalanced by elevated antioxidant defense enzymes such as catalase and superoxide dismutase. Excess ROS in the cardiomyocyte results in further ROS production, mitochondrial DNA damage, lipid peroxidation, post-translational modifications of proteins and ultimately cell death and cardiac dysfunction. Furthermore, ROS modulates transcription factors responsible for expression of antioxidant enzymes. Lastly, evidence exists that several pharmacological agents may convey cardiovascular benefit by antioxidant mechanisms. As such, increasing our understanding of the pathways that lead to increased ROS production and impaired antioxidant defense may enable the development of therapeutic strategies against the progression of DbCM. Herein, we review the current knowledge about causes and consequences of ROS in DbCM, as well as the therapeutic potential and strategies of targeting oxidative stress in the diabetic heart.

Keywords

Diabetes; Diabetic cardiomyopathy; Diabetic heart; Oxidative stress; Reactive oxygen species; Mitochondria

[*]Corresponding author. Medical University of Graz Division of Cardiology, Auenbruggerplatz 15, 8036, Graz, Austria. heiko.bugger@medunigraz.at (H. Bugger).

1. Introduction

Diabetes mellitus (DM) has evolved as an pandemic that currently affects 463 million people worldwide and is projected to increase to 700 million by 2045 [1]. Estimates suggest that over half of people living with DM are even undiagnosed [1]. Despite numerous advances in care and intervention in subjects with DM, cardiovascular (CV) disease remains the leading cause of morbidity and mortality in these patients, mainly related to increased incidence and severity of coronary artery disease (CAD) and myocardial infarction, and consequently increased development of heart failure (HF). Patients with DM have approximately 75% increased risk of CV disease-related death or hospitalization for HF compared to patients without DM [2]. The risk for HF development in DM subjects is increased by two-fold [3,4], and the 1-year mortality due to HF in patients with DM is approximately 1.5-fold greater compared to those without DM [5]. Conversely, the prevalence of DM is four-fold higher in patients with HF compared to those without HF [3,6]. Thus, there appears to be a bidirectional association between HF and DM, where one increases and worsens the prognosis of the other [7,8].

2. Diabetic cardiomyopathy

Although CAD and hypertension are increased in patients suffering from DM, population-based studies have demonstrated that the increased HF risk in DM cannot be solely attributed to these comorbidities [34]. Thus, the term diabetic cardiomyopathy (DbCM) has been coined. DbCM has been defined as ventricular dysfunction in DM subjects in the absence of CAD, hypertension, valvular heart disease, congenital heart disease, and classical causes of cardiomyopathy, or the increased vulnerability of diabetic hearts to fail in the presence of concomitant stress [8–11]. DbCM is characterized by concentric hypertrophy, increased left ventricular (LV) mass, increased ventricular stiffening and impaired relaxation, reflecting a clinical phenotype of diastolic dysfunction that precedes LV dysfunction. At this stage in its pathophysiology DbCM shares features of HF with preserved ejection fraction (HFpEF) [12–15]. It has yet to be confirmed whether DbCM as defined above inexorably progresses to heart failure with reduced ejection fraction (HFrEF) over time [8,10]. Given the resistance of rodents to the development of atherosclerosis and hypertension in the absence of predisposing genetic manipulations [16], studies in rodent models of DM reporting impaired systolic function support the concept that DbCM could potentially predispose to, or may cause HFrEF [17–19]. Both rodent models of type 2 diabetes (T2D), including leptin-deficient *db/db* mice, obese *ob/ob* mice and Zucker diabetic fatty rats, as well as models of type 1 diabetes (T1D), elicited systolic cardiac dysfunction in several studies. Even though some studies have failed to show major cardiac contractile defects as a result of DM in animal models [20–22], this could be related to differences in methodology, gender, age, disease duration and model of DM (reviewed in Ref. [23]). Of note though, despite the absence of systolic dysfunction, many of these models may nevertheless exhibit diastolic dysfunction [24–26].

3. Oxidative stress

The “redox state” is determined by the balance between production of ROS and their removal by the antioxidant defense system. The term ROS includes: the free radicals, superoxide $(O_2^{\bullet -})$ and hydroxyl ($OH^{\bullet}$); the non-radical, hydrogen peroxide (H_2O_2); and the result of the reaction of superoxide and nitric oxide (NO), peroxynitrite ($ONOO^{\bullet -}$). Production and degradation of ROS reflects the normal physiology of many cells; however, when an imbalance occurs from excessive ROS generation and the inability for these to be degraded, this is defined as “oxidative stress”.

In the cardiomyocyte, ROS may be generated in the mitochondria at the electron transport chain (ETC), by monoamine oxidase (MAO) or calpain; by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) and xanthine oxidase (XO) and uncoupling of nitric oxide oxidase (NOS) (Fig. 1 A). This production of ROS is counterbalanced by a complex antioxidant system which detoxifies ROS in order to maintain homeostasis (Fig. 1 B). However, under conditions of pathological stress, excessive ROS production and/or inadequate ROS detoxification may result in ROS-induced damage to DNA, proteins and lipids, leading to irreversible cell damage and death, ultimately resulting in cardiac dysfunction. As such, generation and detoxification of ROS must be tightly regulated in order to prevent oxidative damage, or reductive stress if these pathways are hyperactivated. Importantly, the causal role of oxidative stress in diabetic heart disease is supported by antioxidant interventions to scavenge ROS, which limit or prevent cardiac dysfunction in animal models of DM [27–31].

The most well-studied markers of oxidative stress include the products of lipid peroxidation, malondialdehyde (MDA) and 4-hydroxynonenal (HNE), and the indicator of protein nitration, 3-nitrotyrosine (3-NT). MDA is generated via peroxidation of lysine residues by polyunsaturated fatty acids (FAs) and is commonly measured via calorimetric reaction with thiobarbituric acid (TBA) using a TBA reacting substances (TBARS) assay. HNE is a stable and reactive lipid peroxidation product, generated in a secondary reaction following initial peroxidation of polyunsaturated FAs. Interaction of HNE with macromolecules (e.g., proteins) is easily measured by immunoblotting or ELISA using specific antibodies. Protein nitration occurs when peroxynitrite or nitrogen dioxide (NO_2) react with susceptible tyrosine residues and is most accurately measured by tandem mass spectrometry (MS/MS) in combination with high-performance liquid chromatography (HPLC) but can also be quantified by chromatography or immunohistochemical assays. Other indicators of oxidative stress include oxidized DNA molecules (i.e., modification of guanine [32]), protein modifications (e.g. carbonylation) [33] or modified polyunsaturated FAs (i.e., 8-isoprostane; 8-*iso* $PGF_{2\alpha}$) [34].

4. Oxidative stress in DbCM

Myocardial oxidative stress is a common observation in animal models of DM (Table 1). The most well-characterized animal model of T1DM is generated by intraperitoneal injection of the pancreatic betacell toxic glucosamine-nitrosourea antibiotic, streptozotocin (STZ), which induces hyperglycemia in mice, rats and guinea pigs. Both diastolic and

systolic dysfunction occurs depending on the duration of diabetes and these functional changes have been correlated with increased cardiomyocyte ROS levels [35–38]. STZ-induced diabetes is characterized by increased lipid peroxidation [24,35,36,39], nitrotyrosine [24], carbonyl protein content [40] and reduced GSH [25,35, 41]. Elevated oxidative stress in STZ models is thought to be related to reduced ETC enzymatic activities [24,38,42], whereas the activity of specific detoxification enzymes has been reported to be increased [39, 43,44] or unaltered [35,39,44]. Other rodent models of T1D include the transgenic OVE26 mice, the insulin-dependent spontaneously diabetic BB Wistar rats (ISDBB), the Akita diabetic mouse, and alloxan (ALX)-induced diabetic mice. Of these, OVE26, ISDBB and ALX-induced T2D mice have been reported to display increased markers of lipid peroxidation, tyrosine nitration and protein carbonylation [45–47], in addition to increased expression of antioxidant defense systems [31, 48–50] in conjunction with impaired mitochondrial respiration [48]. Interestingly, others have observed reductions in lipid peroxidation in ALX-induced T1D [51] and in contrast to the other models, the Akita mouse model has decreased mitochondrial hydrogen peroxide generation and no induction of antioxidant defense pathways [52,53].

Rodent models of T2D present with a range of phenotypes depending on the presence or absence of obesity, mode of diabetes induction, and duration of hyperglycemia. The db/db mouse is a severely hyperglycemic genetic model of obesity, insulin resistance and T2D, which presents with altered myocardial substrate use and reduced myocardial efficiency [54]. Similar to the db/db mouse, the male Zucker diabetic fatty (ZDF) rat spontaneously develops T2D and obesity. However, female ZDF rats require the addition of high-fat feeding to induce diabetes [55]. Db/db mice have elevated myocardial superoxide, hydrogen peroxide and peroxynitrite production [56,57]. Furthermore, both db/db mice and ZDF rats display increased lipid peroxidation and protein carbonylation levels [57–60]. Similar to T1D, these models also present with increased activity of antioxidant enzymes, such as CAT and MnSOD, and with impaired ETC activity and mitochondrial uncoupling [57,60]. High-fat or fructose feeding may also be used to induce obesity and/or diabetes and have been found to increase hydrogen peroxide, nitric oxide production [61,62], lipid peroxidation and nitrotyrosine [63], respectively. Studies using a combination of high-fat feeding and a single low-dose injection of STZ demonstrated an increase in mitochondrial ROS production before onset of impaired insulin secretion both in rodents [64] and a large animal model [65], suggesting that metabolic derangements that precede the onset of overt DM may already be sufficient to increase ROS and to contribute to the development of DbCM. Lastly, the Goto-Kakizaki (GK) rat is a unique model of insulin-resistance without obesity, characterized by mild diastolic dysfunction and susceptibility to oxidative stress [66–68]. However, based on the fact that lipid peroxidation is increased [68] while mitochondrial function is preserved in this model, it has been suggested that factors associated obesity and insulin resistance could be a critical mediator of the development of mitochondrial dysfunction, rather than hyperglycemia per se [69].

There exists a large body of literature on preclinical trials of antioxidants that report therapeutic benefit of ROS reduction on diabetic complications, implying that antioxidants may be a viable therapeutic option to reduce the consequences of chronic oxidative stress in diabetes. Importantly, antioxidant vitamin supplementation has been largely insufficient in

showing positive outcomes in clinical trials of CVD, despite their success in preclinical studies [70–73]. As such, new research is focused on fine-tuning the administered dose, as well as optimizing the delivery. However, it should be noted that while evidence of oxidative stress has been reported in multiple organs in humans that exhibit well-characterized diabetes complications, only few data are available regarding oxidative stress in human DbCM (Table 1). Despite the limited number of studies, increased mitochondrial hydrogen peroxide emission, increased mitochondrial ROS levels, and increased levels of HNE-adducts have been consistently observed in atrial tissue of diabetic patients in these studies [74–77]. Although all of these data have been generated from atrial tissue, they are highly suggestive that oxidative stress could also occur in LV tissue of human DbCM. Nevertheless, in order to improve our understanding since clinical trials using antioxidant therapies have failed thus far, a more in-depth analyses of ROS generation and ROS-induced damage are needed, in particular from LV tissue of patients matching the definition of DbCM. Moreover, the development of reliable biomarkers for myocardial oxidative stress, would significantly advance the field.

5. Sources of ROS in diabetic cardiomyopathy

There are several principal sources of ROS generation within a cardiomyocyte that may contribute to oxidative stress in cardiac disease states. Several cytosolic enzymes, such as NADH oxidases (NOX), xanthine oxidase (XO) and uncoupled nitric oxide synthase (NOS), produce ROS during their catalytic activity and alterations in cytosolic ROS generation that may contribute to the pathophysiology myocardial dysfunction in diabetes has been described [78]. However, mitochondrial sources of ROS are thought to represent the major ROS burden in the context of diabetes. Most notably, the electron transport chain (ETC), $p66^{Shc}$, and monoamine oxidase (MAO) are considered the major sources of ROS formation in mitochondria (Fig. 1A). However, given that direct comparison of cytosolic versus mitochondrial sources of ROS production in the diabetic heart are limited, it is difficult to evaluate the relative contributions of each ROS-producing enzyme. Furthermore, it should be noted that while mitochondrial ROS production is elevated in the T2D heart, this may not be the case for several models of T1D [44,79, 80], highlighting important differences that may contribute to the development of DbCM. Nonetheless, literally all of these have been shown or at least been implicated in the development of oxidative stress in DbCM, as schematically illustrated in Fig. 2. The underlying evidence (Table 2) will be presented and discussed in the following paragraphs.

5.1. Mitochondrial electron transport chain (ETC)

The ETC, comprising protein complexes I-IV and the electron transfer carriers, ubiquinone and cytochrome *c*, is the main site of ATP production during oxidative phosphorylation (OXPHOS). Under physiological conditions, electrons are tightly coupled with ATP production, but ROS are nonetheless formed at the ETC from low levels of electron leak (0.2–2%), driven by the activity of ETC complexes, particularly at complex I and III [81]. Brownlee's group provided strong evidence that under hyperglycemic conditions, ROS in vascular endothelial cells are derived from the mitochondrial ETC, as evidenced by an inhibition of increased ROS production by treatment with an uncoupling agent or a complex

II inhibitor [82]. Numerous other studies demonstrated that mitochondrial ROS generation is also increased in cardiomyocytes under hyperglycemic or diabetic conditions, likely generated at the ETC, although the relative contribution of each OXPHOS complex remains a matter of debate [42,57,80]. ADP-stimulated, coupled respiration with electrons delivered via complex I or II is decreased in STZ-diabetic rats, however the amount of ROS generated per oxygen consumed was clearly increased, suggesting that impaired ETC complex activities are involved in increased ROS generation in cardiomyocyte mitochondria [83]. Similarly, succinate-driven ROS production in the presence of the complex V inhibitor, oligomycin, was increased in mitochondria isolated from db/db mouse hearts, and partial inhibition of ROS production following inhibition of complex I with rotenone suggested that ROS may be generated both at complex I but also at other ETC complexes [57]. It is widely speculated that decreased expression or altered composition of OXPHOS subunits observed in models of T1D [84,85] and T2D [57,60] may contribute to increased ETC-related ROS formation, since conditions that impair electron flow through the ETC may increase the likelihood that electrons slip non-specifically from the ETC to generate ROS, as occurs during ischemia reperfusion or heart failure [86].

Of note, complex I or II as a source of ROS-dependent cardiomyocyte dysfunction has also been observed following high glucose (HG)-stimulation of cardiomyocytes from diabetic OVE26 mice [31]. The observation that HG induces ROS production in cardiomyocytes from T2D but not wild-type mice implicates the diabetic milieu in predisposing cardiac mitochondria to ROS production [31]. In fact, a pronounced remodeling of mitochondrial proteins, including ETC subunits, has been observed in hearts of OVE26 mice using 2-D gel comparative proteomics, potentially revealing the biochemical basis for the predilection to ROS production [48]. Thus, it could be hypothesized that hyperglycemia may induce ROS production in diabetes as a result of acquired defects in the ETC. In addition, mice with cardiomyocyte-selective deletion of the insulin receptor demonstrated decreased expression of OXPHOS subunits, associated with increased mitochondrial ROS generation [57,85]. Importantly, the other systemic metabolic perturbations that typically accompany DM are absent in this model, including hyperglycemia. This observation suggests that impaired cardiomyocyte insulin signaling may induce mitochondrial proteomic remodeling, which may increase ROS generation even in the absence of hyperglycemia. Given that impaired cellular insulin signaling often precedes DM, it is possible that the onset of diabetes with concomitant hyperglycemia may further increase ROS generation in the diabetic heart. This hypothesis was partially supported by observations made by Montaigne et al., who observed decreased complex I activity in obese, diabetic individuals, but ROS production was only increased in diabetic hearts [77]. Niemann et al. observed decreased expression of complex I subunits and enzymatic activity of complex I, accompanied by increased protein carbonylation and oxidative DNA damage in right atrial tissue of obese and insulin resistant (but not diabetic) subjects, suggesting that mitochondrial proteomic remodeling during insulin resistance may be sufficient to induce increased ROS [87]. Partial inconsistencies among studies, suggest that a certain degree of ETC remodeling may be necessary to increase ROS generation. These changes are not only determined by obesity and impaired insulin signaling, but also by genetic predisposition and other, maybe unknown, mechanisms. Mechanisms that modulate OXPHOS expression during diabetes development

may differ between subjects, but may include protein degradation, transcriptional regulation, altered protein composition, altered mitochondrial quality control, and others [88].

Given that the diabetic heart predominantly oxidizes FAs, it is also of interest that a cardiomyocyte-specific increase in FAs due to overexpression of ACSL1 has been shown to markedly increase mitochondrial ROS generation at the ETC, suggesting that DM-associated hyperlipidemia and/or the shift in substrate oxidation towards increased FA oxidation may contribute to increased mitochondrial ROS generation at the ETC [89]. Of note, this ROS generation occurred using succinate or palmitoyl-carnitine as a substrate, but not if glutamate was used, and ROS generation was completely blocked by rotenone, suggesting that ROS generation was predominantly driven via complex II. Thus, in addition to hyperglycemia and impaired insulin action, increased myocardial lipid utilization may also contribute to increased mitochondrial ROS generation in the diabetic heart.

5.2. $p66^{Shc}$

Another important source of ROS production is a protein of the Src homology 2 domain and collagen-homology region (Shr) family, $p66^{Shc}$. This cytosolic protein partially translocates to the mitochondria [90], where it catalyzes the electron transfer from cytochrome *c* to oxygen, thereby promoting production of hydrogen peroxide [91]. In addition, $p66^{Shc}$ can activate NOX and inhibit synthesis of antioxidant enzymes, thus further promoting the development of oxidative stress [92]. Importantly, it is believed that persistent transcription of $p66^{Shc}$ occurs as a result of epigenetic signals, such as DNA hypomethylation and H3 acetylation, regulated by miR-218 and miR-34a. Interestingly, mice lacking $p66^{Shc}$ are protected from the consequences of ROS on vasculature in T1D and T2D [93,94]. Of note, upregulation of $p66^{Shc}$ in the diabetic heart has been associated with oxidative stress and left ventricular dysfunction, which were reversed upon gene silencing of $p66^{Shc}$ [95]. Furthermore, ablation of $p66^{Shc}$ expression in cardiac progenitor cells (CPC) and myocytes prevented ROS-mediated tyrosine nitration and 8-OHdG, thereby preserving cardiac gross morphology and function following diabetes [96].

5.3. Mitochondrial monoamine oxidases (MAOs)

The mitochondrial flavoenzymes, monoamine oxidases (MAOs) generate hydrogen peroxide and reactive aldehydes as by-products during the oxidation of monoamines. Recently, MAOs have been implicated as a source of oxidative stress in the heart [97] and have gained traction as a possible target in the treatment of various cardiovascular diseases [98]. Both MAO-A, MAO-B are expressed in the rodent heart, but MAO-A, the major isoform [99], is significantly induced in the myocardium of T1D rats [100]. Under hyperglycemic conditions, enhanced mitochondrial MAO-dependent production of hydrogen peroxide precipitates mitochondrial permeability transition pore (MPTP) opening contributing to mitochondrial dysfunction and impaired endoplasmic reticulum (ER) homeostasis [101]. Pharmacological inhibition of MAO-A, using the specific inhibitor, clorgyline [100] or pargyline [101], attenuated STZ-induced oxidative stress and activation of the ER stress/unfolded protein response (UPR) [100,101]. Importantly, this was associated with reversal of diastolic dysfunction, normalization of cardiac electrical activity and reduced cardiac apoptosis and fibrosis. Altogether, these findings suggest that MAO-induced ROS formation

leads to mitochondrial dysfunction and ER stress, that may contribute to the development of DbCM and may offer a promising target for therapeutic intervention. It should be noted that basal expression of MAO is relatively low in the human heart [102] which limits the translatability of findings drawn from studies in animal models. However, it has been reported that increased activity and expression of both MAO isoforms are elevated in the ventricles of end-stage ischemic failing hearts [103]. Therefore, although MAO expression is similar in right atrial appendages of coronary patients with/without diabetes [104], the effect of diabetes on MAO in the human ventricle is relatively underexplored.

5.4. Mitochondrial calpains

Calpains belong to a family of Ca^{2+}-dependent thiolproteases that have been linked to oxidative stress [105]. The essential regulatory subunit is encoded by *capn4*. The most well-characterized isoforms include calpain-1 and −2, and are ubiquitously expressed in the normal heart. Hearts from T1D [106,107] and T2D [108] mice, display significantly increased calpain activity. Particularly, hyperglycemia [106, 107] and high fat feeding [108] increase calpain activity without altering protein expression of calpain-1 or −2. Importantly, calpain-1 has been directly implicated in superoxide generation via impaired ATP synthase activity [109] and its activation is thought to be mediated by the NOX subunit, $gp91^{phox}$, in cardiomyocytes [106]. Pharmacological inhibition (PD150606, calpain inhibitor-I or III) of calpain activity or overexpression of the endogenous inhibitor, calpastatin, prevent apoptosis induced by hyperglycemia [106,107]. In addition, inhibition of calpain activity prevents HFD-induced apoptosis *in vivo* and *in vitro*. [108] The pro-apoptotic action of calpain may be mediated by stimulating mitochondrial ROS generation [109]. In support of this, reduction of calpain in both T1DM and T2DM prevents cardiac hypertrophy, systolic and diastolic dysfunction, and fibrosis [107,108]. Lastly, transgenic overexpression of calpastatin, which specifically inhibits calpain-1 and −2, has been found to decrease oxidative stress and thereby improve cardiac remodeling following ischemia-reperfusion injury in the diabetic heart [110]. Although increased calpain activity may contribute to oxidative stress in DbCM, additional studies in diverse models and humans will be required to confirm this.

5.5. NADPH oxidases (NOX)

NADPH oxidase (NOX) plays a crucial role in determining the redox state of the heart. Upon formation of a heterodimer with the lower-molecular-weight subunit $p22^{phox}$, the catalytic unit of NOX becomes the site of $O_2^{\bullet -}$ formation upon electron transfer from NADPH to molecular O_2. Of the seven known NOX isoforms, NOX2 and NOX4 have been well characterized in the heart [111]. Activation of NOX2, but not NOX4, depends on interaction with cytosolic subunits, $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and Rac1. Importantly, NOX enzymes have been implicated in the pathophysiology of many cardiovascular diseases, including atherosclerosis, hypertension and heart failure [112]. Of note, NOX2 and/or its subunits are induced in both T1D [26,30,113] and T2D [56, 114]. Fatty acids may also activate NOX2 via a diacylglycerol – protein kinase A mechanism leading to ROS-induced inhibition of lysosomal activity that impairs autophagy in high-fat fed mice (PMID: 25529920). In addition, HG exposure enhances expression and activity of NOX2, and

induces the expression and translocation of its catalytic subunits in cardiomyocytes [106,115–121]. Importantly, studies with the NOX inhibitor, apocynin, demonstrate beneficial effects against the development of diabetes-induced myocardial dysfunction [122]. Deletion or inhibition of NOX2 prevents palmitate-induced ROS production and cardiomyocyte mitochondrial dysfunction [123]. In addition, Rac1 was implicated in STZ-induced myocardial remodeling and dysfunction [124], which may be a result of hyperglycemia-induced cardiomyocyte apoptosis [121]. Furthermore, NOX4 expression is elevated in diabetic hearts [40,125,126], and inhibition by an antisense NOX4 oligonucleotide improved cardiac function in concert with decreased ROS production [125]. While most knowledge of NOX in DbCM pertains to T1D, evidence exists that attenuation of oxidative stress by the cholesterol-lowering drug, ezetimibe, reduced expression of $gp91^{phox}$ and NOX4 in db/db mice [127]. Thus, targeting ROS-producing enzymes such as NOX may have therapeutic potential in both T1D and T2D.

5.6. Xanthine oxidases (XO)

XO as a source of ROS reacts with molecular oxygen to produce superoxide and hydrogen peroxide leading to oxidative stress in diabetes. XO protein expression is significantly elevated in the atrium of STZ-induced T1D rats [128], and XO activity was increased in both ALX-induced T1D rats [45] and fructose-fed diabetic mice [129]. Importantly, increased XO enzymatic activity was accompanied by increased oxidative stress [45]. Of importance, inhibition of XO with allopurinol has been shown to reduce oxidative stress in pre-clinical models of T1D, thereby inhibiting diabetes-induced cardiac remodeling, left-ventricular dysfunction and atrial electrophysiological abnormalities [128,130,131]. Interestingly, attenuation of oxidative stress by allopurinol may be mediated by activation of the NRF2/HO-1 pathway [132]. Allopurinol also inhibits superoxide formation by aortic rings in rabbits with alloxan-induced T1D [133]. While the XO inhibitors, oxyopurinol and allopurinol, may provide CV benefits in patients with chronic heart failure or following myocardial infarction [134,135], their therapeutic potential in the setting of DbCM remains to be investigated. Given that allopurinol prevented an increase in lipid hydroperoxides in the plasma of diabetic patients and that it mediates the above mentioned CV benefits, allopurinol treatment may be a promising therapy to attenuate CV disease in particular in diabetes and deserves to be evaluated in clinical studies [133].

5.7. Uncoupling NO oxidases (NOS)

Under normal physiological conditions, nitric oxide synthases (endothelial, eNOS; inducible, iNOS; neuronal, nNOS) consume NADPH and O_2 to convert L-arginine to L-citrulline, generating nitric oxide and NADP as by-products. However, depletion of the necessary cofactor, tetrahydrobiopterin (BH4) upon oxidation and/or reduced synthesis may lead to reduction of O_2 to superoxide, a process called uncoupling of NOS [136,137]. Accumulating evidence suggests that under hyperglycemic conditions, the uncoupling of NOS generates more superoxide and less NO, thereby contributing to myocardial dysfunction [138]. Particularly, eNOS may promote oxidative stress by competing with the catalytic activity of SOD or by promoting formation of peroxynitrite, which generates protein nitrotyrosine [139]. Conversely, hydrogen peroxide was reported to modulate NOS, specifically in cardiomyocytes [140]. Reduced nitric oxide levels are not only detrimental

for myocardial function [141,142], but further oxidative stress results upon the generation of peroxynitrite by the reaction of superoxide and NO. While nNOS is believed to be the primary nitric oxide producer in the diabetic cardiomyocyte [143], deletion of iNOS, but not nNOS or eNOS, was sufficient to repress markers of oxidative stress in the diabetic heart [144]. Importantly, the beneficial effects of sepiapterin, a precursor of BH_4, on left-ventricular function in STZ-induced T1D is thought to be mediated via enhanced iNOS-derived nitric oxide [144]. Interestingly, both the unspecific NOS inhibitor, L-NAME, and the specific nNOS inhibitor, L-VNIO, partially restore the positive ionotropic effect observed in T1D hearts [143]. Studies with the nitroxyl donor, Angeli's salt (AS), have highlighted the role of uncoupled NOS in hyperglycemia-induced cardiomyocyte damage both *in vitro* and *in vivo. Specifically, treatment with AS significantly reduced myocardial ROS and improved cardiac function in STZ-induced T1D mice, without affecting hyperglycemia*. [145] Importantly, this benefit was mediated by induction of the caveolin-3/eNOS complex to reduce cardiomyocyte hypertrophy and apoptosis [145].

6. Metabolic alterations driving ros-production in diabetic cardiomyopathy

DM is characterized by a perturbation of the systemic metabolic milieu that induces metabolic alterations as well as cellular injury in multiple tissues, including the heart. In T2D, obesity-associated systemic insulin resistance, mainly affecting skeletal muscle, adipose and liver tissue, contributes to increased secretion of insulin and hyperinsulinemia. Hyperlipidemia mainly results from increased lipolysis due to impaired insulin sensitivity of adipose tissue, as well as from hepatic overproduction of TG-rich lipoproteins. Hyperglycemia develops when insulin secretion by pancreatic beta cells is insufficient to offset impaired glucose uptake of insulin-sensitive tissues and increased hepatic gluconeogenesis. In contrast, T1D primarily results from destruction of pancreatic beta cells and thus insulin deficiency, mainly induced by environmental factors and genetic susceptibility. All of these metabolic alterations have been proposed to contribute to oxidative stress in DbCM, on the level of cardiomyocytes, endothelial cells, and the crosstalk between cardiomyocytes and endothelial cells [146,147].

6.1. Hyperglycemia

Glucotoxicity due to excess serum glucose levels has been implicated in many cardiac alterations in diabetes, including generation of AGE, fibrosis, maladaptive inflammatory signaling, O-Glc-NAcylation, and impaired Ca^{2+} handling. Importantly, hyperglycemia is also known to stimulate ROS production in the diabetic heart by diverse mechanisms, including through NOX, XO and NOS [132,145,148]. High concentrations of glucose have also been shown to trigger synthesis of superoxide, nitric oxide, peroxynitrite and AGE by mechanisms such as increased polyol pathway flux, increased hexosamine pathway flux, and activation of protein kinase [82,149–151]. Many of these pathological pathways may be activated by ROS stemming from mitochondria [82]. Furthermore, several interventions, including inhibition of the ETC complex II and overexpression of MnSOD, have been shown to prevent hyperglycemia-induced ROS production [82]. Moreover, a recent study showed that several long noncoding RNAs may play an essential role in high glucose-induced cardiomyocyte oxidative stress [152]. Altogether, these findings suggest that

exacerbation of ROS production in the diabetic heart due to hyperglycemia may result in oxidative stress, which in turn may contribute to the development of cardiac abnormalities in diabetes.

6.2. Hyperlipidemia

The diabetic heart has increased preference for FA as a source of energy, mainly driven by systemic hyperlipidemia and decreased myocardial glucose utilization due to impaired myocardial insulin action or reduced glucose uptake [153,154]. Circulating FAs are taken up by the heart by simple diffusion across the plasma membrane via FA transporters. Inside the cell, FAs are utilized for membrane structure, energy metabolism, and as signalling molecules. However, overload of acyl-CoAs generated from an excess of FAs in the diabetic myocardium can lead to cytosolic and mitochondrial ROS overproduction [155]. Particularly, the delivery of reducing equivalents, NADH and $FADH_2$ resulting from oxidation of fatty acids leads to generation of ROS in the ETC [156,157]. Of note, long-term exposure to the FA, palmitate, promotes ROS production and has been shown to cause increased mitochondrial fission [89]. In addition, accumulated acyl-CoA in the diabetic heart can be recycled to triglycerides or assimilated into complexes, such as ceramides. Several diabetic rodent models have implicated elevated ceramide content and myocardial ROS in the development of cardiac dysfunction [158]. Ceramides are also thought to directly inhibit complex III, thereby generating ROS and inflammation [159]. Lastly, FAs can also regulate lipid homeostasis and oxidation by interacting with members of the nuclear receptor family, such as peroxisome proliferator-activated receptors (PPARs), which may also modulate expression of NOX subunits and their role in contributing to oxidative stress [160].

6.3. Insulin resistance

In many models of T2D, increased ROS levels and evidence of myocardial oxidative stress are associated with impaired insulin-stimulated cardiomyocyte glucose utilization, raising the possibility that impaired cardiac insulin sensitivity could contribute to oxidative stress [161–164]. Myocardial insulin resistance is thought to contribute to functional and structural alterations in the heart and may result from effects that are secondary to systemic metabolic disturbances in insulin resistant states (hyperinsulinemia, hyperglycemia, hyperlipidemia), and/or from intrinsic perturbations of insulin signaling in myocardial tissue [154]. Impaired myocardial expression and translocation of GLUT4 are initial alterations that limit myocardial glucose uptake, and that may occur prior to any defect in the ability of insulin to increase PI3K and Akt signaling, both in animal models and humans with T2D [165,166]. While potential contributions due to accompanying metabolic abnormalities such as hyperglycemia, hyperlipidemia and hormonal changes cannot be clearly distinguished in many models of insulin resistance, obesity and T2D, mice with cardiomyocyte-restricted deletion of the insulin receptor strongly suggested that impaired glucose uptake, mitochondrial dysfunction, and contractile defects may be directly related to impaired cardiomyocyte insulin action [85,167,168]. Of note, these mice also exhibited increased mitochondrial ROS production, potentially related to repression of mitochondrial ETC proteins [85,168]. In addition, it has been proposed that superimposing diabetes may further increase mitochondrial ROS generation since induction of FAO capacity by diabetes may increase the delivery of reducing equivalent to the ETC, thus resulting in increased electron

leakage and ROS generation [85]. Thus, the myocardial metabolic adaptations to impaired cardiomyocyte insulin signaling may predispose the heart to increase mitochondrial ROS generation. Furthermore, mice lacking GLUT4 display elevated expression of the NOX isoforms, NOX1 and NOX2 [169], and antioxidant treatment using TEMPOL reversed this upregulation.

6.4. Hyperinsulinemia

An underappreciated mechanism that may contribute to cardiac ROS production is hyperinsulinemia itself. In response to insulin resistance, the pancreas attempts to compensate by secreting additional insulin [170]. A well described observation is that prolonged hyperinsulinemia increases myocardial mass and decreases cardiac output [171,172]. In several models exhibiting hyperinsulinemia, an association with increased myocardial ROS has been observed, e.g. high sucrose diet [173]. While the mechanisms by which hyperinsulinemia may induce ROS remain largely to be elucidated, NOX is thought to be activated by insulin [174,175] and may thus represent a candidate mechanism that may induce ROS production in diabetic hearts.

7. Antioxidant defense system in diabetic cardiomyopathy

Generation of ROS during cellular metabolism is further controlled by the antioxidant defense system consisting of both enzymatic and non-enzymatic antioxidants [176]. This includes the enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and redoxins (Fig. 1 B) and the non-enzymatic antioxidants, vitamin E, β-carotene, and vitamin C. Based on the fact that antioxidant activities are low in the heart compared to other organs [177], the heart is rather susceptible to oxidative damage. Thus, it is not surprising that expression of many antioxidant enzymes is significantly altered in DbCM (Fig. 2; studies summarized in Table 3). Reports of the levels of antioxidant expression and/or activity in diabetic hearts are largely based on studies in rodent models, highlighting a need for a better understanding of ROS producing and ROS scavenging systems in human DbCM. Importantly, according to a study of human left ventricular tissue, the catalytic activity of GSH-Px is over 10-fold higher than that of CAT, MnSOD or Cu/ZnSOD, which may highlight the relative contribution of each component of antioxidant defense in the human myocardium [178].

7.1. Superoxide dismutase (SOD)

SODs, including MnSOD and CuSOD, are enzymes that accept an electron from superoxide and react with water to create hydrogen peroxide. The myocardial expression of SOD proteins has predominantly been shown to be elevated in models of obesity and T2D [57,61], T1D [39], and in a genetic model of lipid-overload, suggesting that increased SOD levels may protect from oxidative stress by detoxification of highly reactive superoxide [89]. MnSOD is thought to be an important antioxidant enzyme in the heart, given its relatively high levels of abundance within mitochondria, and based on the observation that overexpression of MnSOD in the heart is capable of maintaining mitochondrial morphology, enhancing mitochondrial respiration, and improving contractility in the T1D heart [31]. Furthermore, overexpression of MnSOD completely rescued superoxide production and

lipid peroxidation in a genetic model of lipid-overload, thereby restoring mitochondrial cross-sectional area and number [89]. Furthermore, the SOD mimetic, M40403, attenuated lipid peroxidation and DNA damage induced by HG in the perfused heart, and normalized QT interval prolongation and coronary perfusion pressure [179]. Altogether, these findings highlight the critical role of O_2^- in the development of HG-induced cardiac dysfunction and the therapeutic potential of targeting SOD enzymes in the diabetic heart. It should be kept in mind though that a severalfold overexpression of MnSOD (10–20-fold) in overexpression models, as well as application of SOD mimetics, might only indicate that intensive antioxidant treatment might be required to attenuate oxidative stress and damage, and that targeting SOD enzymes could be a useful approach. However, it is not possible to infer from these studies the pathophysiological significance of a lower levels of MnSOD induction by diabetes in the heart.

7.2. Catalase (CAT)

CAT enzymatically detoxifies hydrogen peroxide into oxygen and water, thus neutralizing it. Both increased expression [29,31,44,49,180] and activity [46,181] of CAT have been reported in rodent models of T1D, although little is known about CAT in the context of T2D. The argument that ROS are implicated in DbCM is further supported by the beneficial cardiac effects of CAT overexpression in diabetic models. Cardiomyocyte-specific overexpression of CAT was found to protect mouse hearts against STZ-induced DbCM, partially by suppressing NF-κB-dependent inflammatory responses and associated protein nitration [182]. This may occur as a result of reduced diabetes-induced cardiomyocyte mechanical abnormalities via altered AKT/Fox-O3a/SIRT2 signaling [183]. Interestingly, although overexpression of CAT preserved l cardiac morphology, prevented the contractile defects, and reduced MDA protein modification, it was unable to reverse the slowed Ca^{2+} decay induced by T1D [29]. In terms of T2D, CAT overexpression also protected against defects in cardiomyocyte contractility in agouti mice [29]. Similar to SOD enzymes, myocardial overexpression of CAT induced by diabetes may serve as an adaptive response to protect from the consequences of oxidative stress.

7.3. Glutathione peroxidase (GSH-Px)

Similar to CAT, GSH-Px is an enzymatic antioxidant that receives hydrogen peroxide from SOD and converts it to water, thereby preventing spontaneous formation of hydroxyl. In contrast, expression of several isoforms of GSH-Px appears to be unaltered in models of both T1D [39,46] and T2D [60,184]. As might be expected, forced overexpression of the mitochondria-targeted antioxidant enzyme, phospholipid hydroperoxide glutathione peroxidase 4 (GSH-Px4), improved ETC function and cardiac function in concert with attenuated hydrogen peroxide production and post-translational modifications (i.e. oxidation and deamination) in STZ-diabetic mice [185]. Furthermore, genetically-induced overexpression of GSH-Px1 attenuated diastolic dysfunction, myocyte hypertrophy, and interstitial fibrosis in STZ-diabetic mice [186]. Of note, mice with GSH-Px1 overexpression demonstrated development of hyperglycemia, hyperinsulinemia, and obesity in another study, which was however interpreted as an interference of GSH-Px1 activity with insulin signaling due to over quenching of physiological levels of ROS that are required for insulin

signaling [187]. Thus, in DbCM, GSH-Px may play a less important role in balancing ROS homeostasis.

7.4. Peroxiredoxin (PRX)/Sulfiredoxin (SRX)/Thioredoxin (TRX)

Peroxiredoxins (PRX) are an antioxidant enzyme family which detoxify hydrogen peroxide and peroxynitrite. The expression of PRX3 is decreased and that of PRX5 is increased in the hearts of T1D rats [100, 181], whereas PRX6 was increased in the fructose-fed rats model of prediabetes [63]. However, the precise role of PRX in the development of DbCM remains unclear. Interestingly, induction of PRX3 expression by the flavonoid, quercetin, prevented T1D-induced oxidative stress and cardiac dysfunction [181]. Furthermore, HG-induced mitochondrial oxidative damage was reduced following overexpression PRX3 in cultured cardiomyocytes [181]. When insufficient amounts of reduced PRX are available to deal with peroxides, hyperoxidation of PRX may occur (Prx-$SO_{2/3}H$), causing inactivation of the peroxidase activity [188]. Both HG-stimulated cardiomyocytes and T1D rats exhibit enhanced levels of hyperoxidized PRX [189,190]. However, this process may be reversed and prevented by the activity of sulfiredoxin (SRX), thereby restoring PRX activity. Importantly, overexpression of SRX prevents PRX hyperoxidation in T1D hearts and high-glucose stimulated cardiomyocytes, whereas SRX gene silencing induced ROS generation [189].

The antioxidant activity of PRX3 and PRX5 depend on recycling by the mitochondrial electron donor, thioredoxin (TRX) 2 [191]. Studies showed a decrease in the expression of TRX2 in HG-treated H9c2 cardiac cells and myocardium of STZ-induced T1D rats [190]. Of importance, overexpression of TRX2 reduced mitochondrial oxidative damage and restores ATP production in HG-treated H9c2 cells [190]. Furthermore, inhibition of TRX reductases (TRXR), thereby reducing TRX activity [192], may be sufficient to impair contractility in the isolated heart [190]. TRXR activity was significantly reduced in T1D hearts and activated by genetic deletion of RAC1, suggesting that NOX may regulate the activity of TRXR in diabetic hearts [121]. Lastly, the activity of the major thiol reducing TRX system is controlled by expression of its endogenous inhibitor, thioredoxin interacting protein (TXNIP), which is increased in diabetic human right atrial biopsies and hyperglycemic models [193,194]. The resulting reduction in TRX activity is further associated with increased ROS, all of which was reversed by downregulation of TXNIP in cultured neonatal cardiomyocytes [193]. Furthermore, diabetic mice with CM-specific deletion of TXNIP showed greater resistance to β-adrenergic stress than controls [194], although this was not attributed to reduced ROS in this study.

7.5. Glutathione (GSH)/glutathione disulfide (GSSG)

Glutathione (GSH) is a nonprotein thiol that defends against oxidative stress by donating a hydrogen molecule in the neutralization of hydrogen peroxide. As a result of this reaction, GSH is converted to the inactive glutathione disulfide (oxidized glutathione; GSSG) by glutaredoxins (GRX), which is reversed by the enzyme, *glutathione reductase (GSR)* [195]. *Under conditions of excessive ROS production, the GSH system can become overwhelmed; therefore, the ratio of GSH to GSSG is considered an indication of oxidant levels. In fact*, GSH depletion has been implicated directly in enhanced mitochondrial ROS production

[192]. *Particularly, GSH/GSSG ratio was found to be reduced in human permeabilized atrial myofibers from T2D patients with EF>30%* [74]. *This is further supported by rodent models of T2D which display both low GSH/GSSG* [56,58,68,114] *and high GSR expression*. [60] *Similar results were reported in most* [25,31, 35,41,48,196] but not all *T1D rodent models* [35,49,51]. Particularly, the role of GRX, which is elevated in the serum of hyperglycemic patients, and LV myocardium of T2D rats [197], has not yet been explored. Nevertheless, free radical scavenging by GSH infusion has been shown to prevent the increase in coronary perfusion pressure induced by HG in isolated hearts [198]. Importantly, this was associated with reduced superoxide and nitrotyrosine levels, without any effect on nitric oxide content. GSH supplementation also prevented apoptosis in T1D rat hearts, suggesting that the GSH system may also regulate cell death in the diabetic heart [196]. The therapeutic potential of GSH is supported by studies using the GSH precursor, *N*-acetylcysteine (NAC), which showed that NAC treatment of STZ-induced T1D mice was sufficient to improve cardiac function and reduce cardiac fibrosis in conjunction with reduced ROS generation [199]. Importantly, NAC normalized ROS production and prevented glutathione loss in cardiomyocytes isolated from T1D rats, and this was associated with reduced apoptosis [200]. In addition, NAC treatment of diabetic rats resulted in a significant restoration in the expression levels of several microRNAs (miR-499, miR-1, miR-133a, and miR-133b) in the myocardium, which are otherwise markedly depressed in diabetic cardiomyocytes [201]. Furthermore, the ability of NAC to ameliorate hyperglycemia-induced cardiac injury may be mediated via enhanced endogenous CoQ levels [202]. Based on positive patient outcomes following NAC supplementation [203–205], improving antioxidant capacity may provide an opportunistic approach to treating oxidative stress-induced cardiac injury in diabetic patients [206]. Lastly, glutathione-*S*-transferases (GSTs), which catalyze the conjugation of GSH was reduced [45] and the multidrug resistance protein 1 (MRP1), a transporter implicated in the efflux of GSH was elevated [35], respectively, in T1D hearts, although their precise role in DbCM remains to be elucidated.

7.6. Metallothionine

Metallothionein (MT) is a cysteine-rich regulator of metal homeostasis that has been shown to have potent antioxidant properties against hydroxyl radical and peroxynitrite [207,208]. Of the four MT isoforms, MT-I and MT-II are the major isoforms in the human and animal heart [209]. Chronic overexpression of MT eliminated excess ROS production in diabetic cardiomyocytes [210], thereby alleviating hyperglycemia induced mitochondrial injury [50] and impairments in cardiomyocyte contractility [113]. In addition, elevated MT attenuated ischemic contractility seen in diabetic hearts [50]. MT exerts cardioprotective effects in diabetic hearts by inhibiting GSH depletion, lipid peroxidation and protein nitration, thereby reducing cardiomyocyte death [211]. Importantly, beneficial effects of MT overexpression are thought to occur without alterations in other antioxidants [212], but may be attributed to reductions in tyrosine nitration and resulting impairment of ATP synthase α subunit [213] and/or SCOT [214]. Interestingly, prevention of DbCM by zinc supplementation is believed to be mediated by elevated MT expression [215]. As such, MT could be a potential strategy for prevention of DbCM through suppression of nitrosative damage (further reviewed in Refs. [216–219]).

7.7. Sirtuins

Sirtuins (SIRTs) are NAD^+-dependent deacylases that regulate protein function by removing posttranslational modifications from protein lysine residues. Impaired activity of sirtuins may contribute to mitochondrial defects in DbCM including oxidative stress. Particularly, impaired antioxidant function is a result of reduced levels of the mitochondrial SIRT3, which promotes increased acetylation and thereby inactivation of MnSOD [220]. Importantly, increased protein acetylation in *db/db* hearts is associated with decreased NAD^+/NADH ratio and reduced expression of SIRT3 and its targets, LCAD and MnSOD [221, 222]. In support of this, deletion of SIRT3 elevates ROS production and enhances cardiomyocyte necrosis, thereby reducing mitochondrial membrane potential and aggravating cardiac dysfunction in T1D [223]. Moreover, overexpression of SIRT3 reduced HG-induced hydrogen peroxide production in human cardiomyocytes [224]. Interestingly, the antioxidant effects of the peptide, elabela, in diabetes-induced cardiac injury may be mediated by Foxo3a deacetylation by SIRT3 [225]. Although not well-characterized, reduced SIRT2 expression may also play an important role in DbCM and is reversed along with the beneficial cardiac effects of CAT overexpression [183]. In addition, the novel thiazolo[3,2-a]pyrimidine derivative, LF10, suppressed cardiac dysfunction in T1D upon restoring SIRT1 and preventing MAPK activation [226]. Nonetheless, the exact roles of SIRT1 and SIRT3 in the development of DbCM requires further investigation.

7.8. Nuclear factor erythroid 2-related factor 2 (NRF2)/Heme oxygenase (HO)-1

The nuclear factor erythroid 2-related factor 2 (NRF2) is a master transcriptional regulator that controls the expression of antioxidant genes in response to oxidative stress, thereby playing an important role in cell survival [227]. The activity of NRF2 is primarily regulated via its interaction with Kelch-like ECH-associated protein 1 (Keap1), which promotes ubiquitination/degradation of NRF2 [228]. Under conditions of oxidative stress, NRF2 dissociates from Keap1 and translocates to the nucleus, where it regulates gene expression upon binding to antioxidant response elements (AREs) located in the promoter of its target genes [229]. Cytoprotective genes upregulated by NRF2 include the antioxidant genes, GSH-Px and SODs, as well as the cytoplasmic detoxification enzyme, NAD(P)H quinone dehydrogenase (NQO)-1, and heme oxygenase (*HO*)-1 [230]. Importantly, evidence of oxidative stress in T1D has been attributed to impaired NRF2-induced antioxidant expression [132,231]. In support of this, nuclear expression of NRF2 and its downstream target, HO-1, are reduced following HG-stimulation of human cardiomyocytes [232]. It should be noted that while expression of NRF2 appears to be significantly downregulated in cardiac tissue from diabetic patients [233], HO-1 expression is increased in diabetic patients without heart failure, although this may be attributed to obesity rather than T2D [234]. Interestingly, mRNA content of HO-1 was also increased in STZ-induced diabetic rat hearts [235]. Thus, downregulation of NRF2 might not be an early response to hyperglycemia [236,237], however evidence exists that the NRF2 adaptive response to glucose may be impaired in T2D [238]. Importantly, deletion of NRF2 exacerbated ROS production and increased sensitivity to mitochondrial respiratory complex II inhibition in HG-stimulated cardiomyocytes, and this was associated with impaired cardiomyocyte contractility and increased cellular apoptosis [237]. Furthermore, deletion of NRF2 exacerbated HFD/STZ-

induced cardiac damage, and this was partially reversed by supplementation with sulforaphane (SFN) [239].

Meanwhile, overexpression of HO-1 inhibited oxidative stress, inflammation and apoptosis and enhanced autophagy, thereby ameliorating the pathogenesis of cardiac dysfunction in T1D [240]. Furthermore, the protective myocardial effects of several natural compounds against hyperglycemia-induced oxidative stress have been shown to be dependent on NRF2/Keap1 in diabetes [241–245]. Notably, the beneficial effects of SFN in the diabetic heart appear to be mediated by NRF2-dependent induction of MT [236,239]. However, conflicting reports have also implied that cardiomyocyte-specific overexpression of NRF2 may worsen progression of DbCM, whereas global NRF2 knockout may prevent the progression of cardiac dysfunction in T1D [246]. Based on this, the potential of targeting NRF2 as a therapeutic strategy in diabetes requires further exploration (further reviewed in Refs. [247, 248]).

7.9. Aldehyde oxidases

Mitochondrial acetaldehyde dehydrogenase 2 (ALDH2) is a nuclear-encoded aldehyde oxidase responsible for the metabolism or detoxification of acetaldehyde and other toxic aldehydes. ALDH2 polymorphisms are associated with an increased risk of T2D in female CAD patients [249]. Furthermore, proteomics analysis of hearts from STZ-induced T1D rats revealed significantly decreased expression of ALDH2 [41]. Importantly, overexpression of ALDH2 attenuates HG-induced cardiac injury by suppressing mitochondrial ROS production and inhibiting NLRP3 inflammasome activation [250]. In addition, ALDH2 protects against myocardial dysfunction in diabetes through an AMPK-dependent regulation of autophagy [251]. Moreover, the cardioprotective effects of ALDH2 in insulin resistance-induced cardiomyopathy may involve SIRT3 [252]. These studies suggest potential therapeutic promise of targeting ALDH2 in DbCM, although further studies are required.

8. Consequences of ROS in diabetic cardiomyopathy

ROS has been well-documented to play a causative role in the development of DbCM [253,254]. Regardless of the source of ROS, increased ROS levels may result in damage to nucleic acids, proteins, and lipids, resulting in a plethora of cellular abnormalities that may culminate in cardiomyocyte dysfunction and death in diabetes (Fig. 3). Below, we discuss maladaptive cellular alterations evident in DbCM that have been causally linked to increased ROS and oxidative damage.

8.1. DNA damage

Although there are relatively few proteins encoded by mitochondrial DNA (mtDNA), mtDNA appears to be particularly susceptible to oxidative damage [255]. MtDNA number, as well as expression of proteins that control replication and transcription of mtDNA are elevated in OVE26 T1D hearts [48], but reduced in HFD-induced T2D mice [256]. Oxidation of guanine residues results in the accumulation of 8-hydroxyguanine (8OHG), 8-hydroxydeoxyguanosine (8OHdG), and 8-oxo-7, 8-dihydro-2-deoxyguanosine (8-oxodG) [32,257]. Moreover, 8-oxoguanine glycosylase (OGG-1) is the primary enzyme responsible

for reversing this reaction and may be inactivated in the diabetic heart by 4-HNE modification [59]. Importantly, overexpression of OGG-1 prevented fibrosis and improved contractile function the T1D heart [258]. In addition, HG-induced increases in 8-OHdG are attenuated by enhanced antioxidant capacity [179].

8.2. Lipid/protein peroxidation

In addition to DNA, unsaturated lipids are considered a vulnerable target of ROS. Malondialdehyde (MDA) is a product of lipid peroxidation (LPO) and is a commonly used marker of oxidative stress. Increased levels of lipid peroxides have been reported in the serum of diabetic patients [259–261] and in the heart of both T1D [24,35,39,45,196] and T2D [57,60,61,68,114,241] rodents, although this is not always the case [51,262]. In addition, hyperglycemia and/or hyperlipidemia both increase lipid peroxidation levels specifically in the heart or cardiomyocytes [179,232,240,241]. Importantly, the contribution of ROS to enhanced lipid peroxidation is evident by reduced MDA levels upon targeting ROS-producing enzymes [100,145], activating antioxidant defense systems [29,240], or improving ROS scavenging [27,226]. Of note, enhanced myocardial lipid peroxidation may be a direct consequence of myocardial lipid overload [61,89]. Proteins are also subject to peroxidation (marked by 4-HNE adducts) under conditions of persistent lipid peroxidation, as evidenced in atria of T2D patients [74]. 4-HNE modification of certain mitochondrial proteins, such as the FAD-containing subunit of succinate dehydrogenase [42], may contribute to mitochondrial defects and therefore myocardial contractile dysfunction in diabetes. In fact, inhibition of MAO [101] and induction of PRX-3 [181] were found to reduce cardiac levels of 4-HNE in association with reduced diastolic stiffness. Lastly, another modification shown to be a sensitive index of oxidative stress is 8-isoprostane prostaglandin (8-*iso* $PGF_{2\alpha}$) [34], which is also elevated in the heart of STZ-induced T1D rats [41].

8.3. Nitrotyrosine (3-NT)

In addition to peroxidation, nitration of proteins may also occur, particularly at tyrosine residues (3-nitrotyrosine; 3-NT). This is often used as a biomarker of cardiovascular risk [263], and may be implicated in the development of DbCM [264,265]. While 3-NT is increased in diabetic hearts, this damage can be reversed following inhibition of XO [130], by overexpressing antioxidant proteins [182,213,214], and by CoQ10 supplementation [27,28]. Indeed, reduced nitration of specific enzymes involved in metabolism [214,262,266], contraction and antioxidant defense [47] may be implicated in the impairments of the diabetic heart. Particularly, direct damage of ETC complexes have been characterized in hearts of STZ-induced diabetic rats [42,47]. Notably, enhanced antioxidative capacity by NRF2 may play a protective role in mediating against 3-NT in DbCM [257]. Interestingly, both lipid peroxidation and 3-NT are not increased in hearts of pre-diabetic mice [63], suggesting that this may be a consequence of prolonged metabolic disturbances in long-standing diabetes.

8.4. O-GlcNAcylation (O-GlcNAc)

O-GlcNAcylation (O-GlcNAc) is a specific posttranslational modification at Ser/Thr residues that alters the activity of the target protein. O-GlcNAc-transferase (OGT) and O-

GlcNAcase (OGA) are the enzymes responsible for protein modification by O-GlcNAcylation [267,268]. Interestingly, expression of OGT and OGA appears to be increased in T1D in association with hyperglycemia-induced superoxide production [26]. Moreover, ROS-induced mitochondrial dysfunction in diabetes has been proposed to occur through O-GlcNAc [269]. In addition, subunits of the respiratory chain complexes I, II and IV undergo enhanced O-GlcNAc under HG conditions, in concert with impaired activity of specific proteins that regulate mitochondrial dynamics, optic atrophy 1 (OPA1) [270] and/or dynamin-related protein 1 (DRP1) [271]. Moreover, improper localization of OGT to the mitochondria prevents normal complex IV activity, thereby reducing mitochondrial membrane potential and impairing respiratory function [269,272]. O-GlcNAcylation of CaMKII during diabetes is also thought to impair Ca^{2+}-handling and may be implicated in cardiac arrhythmias and dysfunction [273,274]. Altogether, these modifications induced by altered substrate metabolism and oxidative stress are thought to be toxic, particularly to the cardiomyocyte, and likely contribute to the onset of cardiac dysfunction in DbCM. Key myocardial proteins that are modified by O-GlcNAc and are involved in substrate metabolism and antioxidant defense (amongst other pathways) have been recently reviewed [254].

8.5. Advanced glycation end-products (AGE)

Advanced glycation end-products (AGEs) are the result of non-enzymatic glycation and oxidation of long-lived proteins and lipids, thereby disabling their functional properties [275,276]. Accumulation of AGE and its receptor (RAGE) have been noted to result from enhanced oxidative stress in hearts from pre-clinical models of diabetes [25,277], as well as cardiac tissue of diabetic HF [278] and aortic stenosis [279] patients. This is coupled with increased expression of NOX2 and its catalytic subunits [280]. Importantly, a causative role of AGE in oxidative stress and cardiac injury was recently demonstrated using engineered cardiac tissues (ECTs) as an *in vitro* model of DbCM [281]. Furthermore, AGE have been implicated in the progression of contractile dysfunction in DbCM by mechanisms that include increased Ca^{2+} leak in the heart [282]. Importantly, excess AGE formation is thought to contribute to myeloperoxidase (MPO) pathway induction [283], which will further exacerbate AGE formation [284], thus causing feed-forward-fueled pathological loops that amplify metabolic dysfunction. Furthermore, activation of the AGE/RAGE axis may impair activity of SIRT1 [285,286], although this phenomenon requires additional exploration in the diabetic heart.

8.6. Mitochondrial dysfunction

Since mitochondria are a source of ROS, they are also a major target of ROS damage. Importantly, mitochondrial dysfunction is a characteristic of obesity as well as diabetes/insulin resistance in the human heart [69,87]. Diabetic patients with both diastolic dysfunction [287] and normal [288] cardiac function display reduced cardiac phosphocreatine/ATP ratios, suggesting that mitochondrial energetics is compromised in the diabetic heart. Increased hydrogen peroxide emission is associated with impaired mitochondrial respiratory function in left atrial appendage of T2D patients with CAD [74]. Evidence of increased mitochondrial oxidative stress was associated with increased sensitivity to calcium-induced MPTP opening in atria from T2D patients [74,75].

Mitochondrial bioenergetic dysfunction in diabetes may also result from increased uncoupling [40], which negatively impacts mitochondrial energetics and Ca^{2+} uptake [289]. In addition, impaired removal of damaged, ROS-overproducing mitochondria through mitophagy within diabetic cardiomyocytes has been described [290]. Of note, MT overexpression restored mitochondrial structure which is otherwise disorganized in hearts of OVE26 mice [50]. In addition, overexpression of MnSOD improved respiration and normalized mass in diabetic mitochondria [31]. Furthermore, pharmacological and genetic inhibition of NOX2 prevents palmitate-induced ROS generation, depolarization of the mitochondrial inner membrane and Ca^{2+} overload in isolated cardiomyocytes [123].

8.7. Cell death

Enhanced cardiomyocyte apoptosis in both diabetic humans and rodent models may contribute to diabetic cardiac remodeling and dysfunction [200,291–293]. Evidence of myocardial apoptosis in both T1D [37,100,196] and T2D [294] models includes increased number of TUNEL-positive nuclei, reduced B-cell lymphoma 2 (BCL-2)/Bcl-2-associated X (BAX) ratio, and increased caspase (CASP) signaling (particularly CASP3, CASP9 expression and activity). In addition, *in vitro* exposure of cardiomyocytes to hyperglycemia [224,237,295,296] and increased fatty acids [297,298] will activate apoptosis. Increased cell death is associated with increased 3-NT [299,300], thereby implicating ROS in diabetes-related apoptosis [96,292]. Apoptosis may also result from GSH depletion linking oxidative stress with autophagy induction [196]. While the mechanisms by which hyperglycemia induces cardiomyocyte apoptosis are not fully understood, one proposed mechanism is via calpain-induced superoxide production [109]. Particularly, modulating the calpain/calpastatin system protects cardiomyocytes from apoptotic death in a model of T1D [106]. Interestingly, NOX has also been implicated in cardiomyocyte apoptosis since knockdown of Rac1 or treatment with the NOX inhibitor, NSC23766, significantly inhibited apoptosis and resulted in improved myocardial function of T2D mice [121]. There have been conflicting reports as to the potential role of Nrf2 in mediating diabetes-induced cardiomyocyte apoptosis. While some groups have reported that absence of NRF2 exacerbates [237,257] and overexpression of HO-1 prevents oxidative stress and apoptosis, others have suggested that deletion of NRF2 inhibited apoptosis and the progression of cardiac dysfunction in T1D [301]. Novel opportunities to ablate the formation of ROS and reduce apoptosis have been suggested by results of inhibition of several long-noncoding RNAs (lnRNA) [152]. Taken together, suppression of cardiomyocyte apoptosis by inhibiting upstream activation pathways induced by oxidative stress, may be beneficial in reducing the deleterious effects of diabetes on the heart.

8.8. Cardiac remodeling and dysfunction

Exacerbation of cardiac remodeling and dysfunction in diabetes may represent the clinically most relevant feature of DbCM, which can be considered as a common downstream consequence of numerous mechanisms that may contribute to DbCM, including oxidative stress. Cardiomyocyte loss precipitates replacement fibrosis by activated fibroblasts altering tissue composition and ventricular compliance leading to cardiac remodeling and impaired diastolic function [302]. Pre-diabetic rodents display early manifestation of a mild hypertrophy and diastolic dysfunction, without signs of fibrosis [63,64]. Importantly,

mitochondrial oxidative stress is associated with the progression of diastolic dysfunction in a model HFD-induced T2D [303]. HG induces hemodynamic and electrical alterations characterized by QT interval prolongation and increased coronary perfusion pressure in the isolated working rat heart [179]. A causative role of ROS in the development of DbCM is evident by reversal of diabetes-induced electrical remodeling and prevention of diastolic stiffness by targeting ROS producing proteins or enzymes, such as calpains [107–109], NOX [121,122,124,125], MAOs [100,101], and XO [128,130,132]. Furthermore, enhancements of the antioxidant defense system appear to protect against diabetes-induced impairment in contractile function, as evidenced by overexpression of CAT [29,182], MT [50,113,210,264], mPHGPx [185], and genetic and pharmacological activation of SOD [31,179]. Reversal of impaired contractility and improved calcium handling as a result of these therapies, may be related to increased expression of contractile proteins [304], and reduced tyrosine nitration of metabolic proteins [213,214]. Additionally, loss of NRF2-induced expression of cytoprotective genes may contribute to myocardial structural damage and impaired response to isoproterenol stimulation of diabetic hearts [257]. Altogether, these findings, as well as improved cardiac morphology upon restoring antioxidant status by agents such as NAC [201] and quercetin [181], suggest that attenuating oxidative stress in diabetes may offer a strategic approach to limit cardiac functional abnormalities as a consequence of DbCM.

9. ROS scavengers

Preclinical studies showing beneficial effects of decreasing oxidative stress in the diabetic heart fostered studies in animals and humans to evaluate antioxidant treatment as a useful approach to attenuate diabetic heart disease (Table 4). These approaches include mitochondria-targeted therapies, given the crucial role of mitochondrial ROS in DbCM.

9.1. Mito-TEMPO & MitoQ

Based on inconclusive evidence that antioxidants are sufficiently delivered to the mitochondria to therapeutically target ROS production, mitochondrial targeted antioxidants have been developed. In these compounds, the active antioxidant substance (e.g., TEMPO) is conjugated to the lipophilic triphenylphosphonium cation (TPP^+), which in some cases facilitates an up to 1000-fold accumulation within mitochondria, driven by the negative intramitochondrial charge. As expected, mito-TEMPO passes through lipid bilayers, to scavenge ROS, and to eliminate the increase in ROS production induced by palmitate in cardiomyocytes [123,305] Interestingly, mito-TEMPO treatment improved diastolic function in db/db T2DM mice, and both diastolic and systolic function in STZ-induced T1DM mice [306]. Importantly, this was associated with inhibition of mitochondrial ROS production and oxidative stress, as well as reduced apoptosis. Another TPP^+ conjugated compound is MitoQ (TPP^+ conjugated to ubiquinone), which is reduced to ubiquinol within mitochondria and then acts as an antioxidant at the ETC [307]. Importantly, MitoQ reduces oxidative stress, enhances OXPHOS and prolongs cell survival under hyperglycemic and hyper-lipidemic conditions [308,309]. While protective effects of MitoQ have been established in the diabetic kidney [310] and in the heart following ischemia-reperfusion injury [311], experimental data on treatment effects of this potent antioxidant in models of DbCM are

currently lacking. Given the safety of MitoQ in humans and its efficacy in attenuating ROS-induced vascular endothelial cell dysfunction in human subjects, studies of this promising compound in the setting of DbCM would be of utmost interest [312].

9.2. Flavonoids

Flavonoids are naturally occurring compounds acting upstream of mitochondria that have shown promise in models of diabetes, potentially as a consequence of their antioxidant properties. Resveratrol, a naturally occurring polyphenol found in grapes, has been shown to elicit protective effects in diabetes, particularly in the heart (extensively reviewed elsewhere [313]). Improved cardiac function following resveratrol treatment of T2D *db/db* mice was associated with reduced 3-NT and improved nitric oxide availability, likely upon reduced iNOS expression, enhanced eNOS-induced nitric oxide production and inhibition of ROS production by NOX [314]. Furthermore, resveratrol is believed to enhance endogenous antioxidant defense [296], thereby protecting cardiomyocytes from hyperglycemia-induced apoptosis [315]. Reducing mitochondrial ROS generation by resveratrol, attenuated cardiac fibrosis, accumulation of reactive lipids, and respiratory kinetics for palmitoyl-CoA in T2D ZDF rats [316]. Attenuation of cardiac oxidative stress by resveratrol has also been attributed to activation of SIRT1 and resulting deacetylation of NFkB-p65 and histone H3 [317]. These beneficial cardiac effects of SIRT1 activation by resveratrol in DbCM may depend on autophagic flux [318]. Furthermore, the natural hydroxylated analog of resveratrol, piceatannol, suppressed the pathological changes induced by oxidative stress in T1D hearts, in an NRF2 dependent manner [244]. The flavonoid quercetin, which is highly abundant in the human diet, accumulates in mitochondria [319] and is thought to have cardiac benefits via reducing oxidative stress. Quercetin reduced oxidative stress in hypercholesterolemic/hyperglycemic rats, thereby restoring cardiac bioenergetics [320]. The beneficial effects of quercetin may be mediated by induction of PRX3 expression [181]. In addition, the active quercetin glucoside, spiraeoside, prevented HG-induced oxidative stress and apoptosis in human cardiomyocytes upon activation of NRF2/HO-1 [232]. Lastly, taxifolin, also known as dihydroquercetin, reduced hyperglycemia-induced ROS and apoptosis both *in vivo* and *in vitro*, possibly by activation of JAK/STAT3 signaling [321]. Thus, modulation of diet composition towards increased flavonoid uptake, or adding flavonoids as a nutritional supplement to the diet, may represent a promising approach to attenuate diabetic heart disease.

9.3. Natural antioxidants

There is growing interest in the use of non-enzymatic antioxidants as a therapeutic approach for cardiovascular diseases. Vitamin E (α-tocopherol) acts as an antioxidant by interrupting formation of lipid radicals, thereby producing a much more stable vitamin E radical [322]. Vitamin C (ascorbic acid) is then responsible for reducing vitamin E back into a functional free radical scavenger [323]. Studies with these natural antioxidants and others have supported therapeutic potential in the context hyperglycemia-induced cardiac dysfunction [324,325]. Particularly, vitamin E supplementation was found to attenuate reduced LV contractility and slowing of LV relaxation upon reducing myocardial 8-*iso* $PGF_{2\alpha}$ and GSSG accumulation in STZ-induced T1DM rats [326]. Treatment of cardiomyocytes with vitamin C prevented STZ-induced oxidative stress and contractile dysfunction [327].

Nonetheless, while these results appear to be promising, the effects of these agents in human studies suggest that vitamin/antioxidant supplementation is insufficient to provide a long-standing clinical benefit on CV outcomes [206,328, 329]. While short-term intra-arterial infusions of vitamin C have been shown to improve endothelial function in both T1D and T2D [330,331], administration of a combination of vitamin C and vitamin E for 6 months improved endothelial function only in patients with T1D but not T2D [332]. In another study, high doses of oral vitamin C did not improve endothelial function in patients with type 2 diabetes [333]. Vasodilatory function and LV function did also not improve in response to 12 months of high dose vitamin E supplementation both in T1D and T2D subjects [334]. In the HOPE trial, which investigated a two-by-two factorial design to receive either 400 IU of vitamin E daily or matching placebo and either ramipril or matching placebo, no beneficial effects were observed on CV events, CV death, or HF [328]. Extended follow-up of this study cohort even demonstrated an increased risk for heart failure or heart failure hospitalization [335]. Finally, lack of an effect of vitamin E supplementation in prevention of major CV events in diabetic subjects was also confirmed by a meta-analysis of 50 studies [329]. Interestingly, vitamin E supplementation was actually found to reduce, rather than increase, total antioxidant capacity [336]. Thus, long term antioxidant treatment with vitamin E and/or C does not seem to lead to any clinical benefit with respect to vascular outcomes or heart failure development, although the effects on the molecular biology of DbCM has not been evaluated. A differential effect between T1D and T2D cannot be ruled out. Explanation for a lack of efficiency may include that the administered dose and duration of supplementation with vitamins may be insufficient to reverse the pathological changes associated with T2D [337–340]. While it has been shown that vitamin C supplementation indeed may not reach similar serum levels in diabetic patients as it does in healthy subjects [333,341], the extended follow-up period in the HOPE-TOO trial with several years of observation argue against a treatment duration issue [335]. Another explanation for lack of efficiency may be that antioxidants, such as vitamin E, may not reach the target cell in humans as they do in animal models despite achieving appropriate serum concentrations. Novel studies have demonstrated the use of nanoparticle-based therapies that enable improved tissue delivery of vitamin E with reduced side effects in the treatment of animals in models of endotoxemia [342]. In addition, improved treatment efficacy due to nanoparticle-mediated drug delivery has been shown for a number of substances, such as cisplatin improving colon cancer chemotherapy or adenosine providing neuroprotection following stroke [343,344]. Although not yet explored in the setting of DbCM, these targeted treatments in general may offer a unique approach to combat the pitfalls experienced using antioxidant therapies in clinical trials to date. Finally, future trials might need to focus on those individuals with clear evidence of oxidative stress. Viability of this approach will require identification of robust biomarkers.

9.4. Coenzyme Q10 & Q9

Coenzyme Q10 (CoQ10) is a cofactor of mitochondrial respiration that acts as an endogenously synthesized lipid soluble antioxidant [345]. Supplementation with CoQ10 attenuates oxidative stress and cardiac dysfunction in rodent models of both T1D [30] and T2D [27] by preventing induction of ROS. Furthermore, chronic CoQ10 supplementation prevented diabetes-induced cardiomyopathy despite impaired cardiac PI3K signalling [28].

Reduced levels of coenzyme Q9 in the T1D heart is postulated to increase its susceptibility to mitochondrial oxidative stress [68], although its therapeutic potential in the treatment of DbCM has not yet been explored. Nevertheless, this compound holds promise of showing protective effects in human diabetic hearts, given that a number of small sized clinical trials reported potential prognostic advantages in subjects with HFrEF treated with CoQ10 supplementation (reviewed in Ref. [346]).

10. ROS scavenging properties of approved drugs used in diabetic individuals

10.1. Sodium/glucose co-transporter 2 inhibitors (SGLT2) inhibitors

Several classes of drugs in routine use in clinical practice might mediate some of their beneficial cardiovascular effects because of antioxidant properties (Table 4). Sodium/glucose co-transporter 2 inhibitors (SGLT2i) block 90% of glucose reabsorption in the kidney, thereby lowering serum glucose levels. In studies investigating the cardiovascular safety of the antidiabetic drugs, SGLT2i have shown considerable reduction in cardiovascular risk in T2D patients with high cardiovascular risk or known cardiovascular disease [347–349]. Although a number of mechanisms have been proposed to underlie these beneficial effects of SGLT2i, specific mechanisms of action remain elusive. Glucose-lowering effects of SGLT2i may be of less importance for these beneficial effects, given that the heart failure risk reduction was similar in heart failure patients, in the presence or absence of T2D [350]. Of note, several studies have highlighted the antioxidant properties of the SGLT2i, empagliflozin, in the heart of T1D [351] and T2D[256,352,353, 354] mice. Interestingly, cytosolic and mitochondrial hydrogen peroxide correlated with stiffness of cardiomyocytes from ZDF rats, and this was reversed following treatment with empagliflozin [352]. Importantly, these improvements are likely a result of improved NO bioavailability and attenuation of eNOS activation, thus resulting in improved endothelial function [352]. This is further supported by reduced cytosolic ROS production in ventricular cardiomyocytes associated with enhanced expression of Ca^{2+}-handling proteins [351]. These beneficial effects of empagliflozin on myocardial structure and function in the diabetic heart have been linked to reduced myocardial oxidative stress mediated by enhanced nuclear translocation of NRF2 and subsequent expression of antioxidant proteins [355]. The attenuation of myocardial hypertrophy and fibrosis, and improvement of cardiac function observed in T2D hearts may be dependent on sGC-cGMP-PKG signaling [353] or Sestrin2-mediated AMPK-mTOR signaling [256]. While empagliflozin has been shown to reduce expression of key enzymes implicated in oxidative stress [256,353], this effect is most likely secondary to reductions in hyperglycemia or other metabolic improvements, rather than upon direct action on ROS-producing or ROS-scavenging proteins. Lastly, empagliflozin treatment reduced cardiomyocytes stiffness and attenuated oxidative stress parameters in human LV biopsies, although these were isolated from non-diabetic patients with diastolic dysfunction [352]. Of note, the ongoing EMPA-HEART trial will further determine the antioxidant potential of empagliflozin in diabetic patients by measuring plasma biomarkers of oxidative stress, such as myeloperoxidase (MPO) [356].

10.2. Glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs)

Another class of antidiabetic drugs that showed beneficial cardiovascular effects in T2D is glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs). Although some large scale clinical trials for GLP-1RAs [357–359] failed to show any cardiovascular benefit in patients with T2D and existing CVD, the LEADER [360] and SUSTAIN-6 [361] trials demonstrated a significantly lower rate of first occurrence of CV death and nonfatal MI and stroke upon treatment with liraglutide and semaglutide, respectively. Pre-clinical studies suggested that cardiac protective effects of GLP-1RAs may be driven by antioxidant effects. The GLP-1RA, exenatide, attenuated ROS production and enhanced antioxidant defense to protect against cardiac dysfunction in both T1D and T2D models [362]. Interestingly, the beneficial effects of exenatide against apoptosis in isolated cardiomyocytes was partially abolished in the presence of the CAT-inhibitor, 3-AT [362], suggesting that the antioxidant effects of this GLP-1RA may be dependent on CAT-mediated ROS-scavenging. Additionally, the GLP-1RA, liraglutide, was also recently shown to ameliorate interleukin (IL)-1β-induced cellular ROS production and NOX4 expression in cultured cardiomyocytes [363]. Furthermore, the novel GLP-1RA, oral hypoglycemic peptide 2 (OHP2), as well as both semaglutide reduced myocardial lipid accumulation, oxidative stress and mitochondrial dysfunction in diabetic hearts [354]. However, results of CV outcome trials of GLP1RAs have not revealed a signal of reduced HF hospitalization [357,358,360].

10.3. Dipeptidyl peptidase-4 (DPP-4) inhibitors

DPP-4 inhibitors which impair the degradation of GLP-1, reducing glucagon and blood glucose levels in diabetes, may also have antioxidant effects in the heart. For example, sitagliptin reduced lipid peroxidation and enhanced antioxidant defense in hearts of T1D rats and this was associated with a reduction in indices of cardiovascular risk [364]. Vildagliptin and sitagliptin both reduced lipid peroxidation, protein carbonylation and ROS production in rodent models of HFD-induced T2D which is associated with improved HR variability and reduced mitochondrial depolarization and swelling [365,366]. Furthermore, the cardiac improvements observed in female T2D mice upon treatment with DPP-4 inhibitor, linagliptin, are similarly associated with reduced markers of cardiac oxidative stress [367]. The beneficial anti-oxidative effects of DPP-4 inhibitors may be due to improved mitochondrial function and increased mitochondrial biogenesis [368] and related to improved autophagy [369]. Interestingly, studies in the kidney have demonstrated that the antioxidant effect of DPP-4 inhibitors may be dependent on an intact antioxidant system [370], although whether this is the case in the diabetic heart has yet to be confirmed. Interestingly, reduced oxidative stress parameters in the diabetic heart are not observed with DPP-4 inhibitors in all models of diabetes [371,372], suggesting the antioxidant potential of DPP-4 inhibition requires addition investigation. Unfortunately, while these preclinical data appear promising, none of the recently published large CV outcome trials demonstrated any significant cardiovascular benefit in patients with T2D.

10.4. Renin-angiotensin-aldosterone system (RAAS)

Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) are commonly used for the treatment of hypertension and to prevent or attenuate diabetic

nephropathy in diabetic individuals. Activation of the RAAS has also been implicated in the pathogenesis of DbCM by promoting loss of CM and myocardial fibrosis [373,374]. ACEi and ARB decrease oxidative stress in diabetic hearts, resulting in reduced apoptosis [37]. Benazeprilat and valsartan, partially protected against STZ-induced cardiac dysfunction by reducing myocardial oxidative stress [375]. Azilsartan significantly reduced oxidative DNA damage and cardiac dysfunction in *db/db* mice [376]. Reduced oxidative stress by olmesartan may be mediated by enhanced expression and/or activation of cardiac NRF2 in T2D OLETF rats [238]. The ACE inhibitor ramipril ameliorates diabetes-related redox imbalance, as evidenced by reduced superoxide, 3-NT content, and NOX expression [30]. Furthermore, the first angiotensin receptor-neprilysin inhibitor (ARNi) to be used clinically, LCZ696 (valsartan/sacubitril), improved ventricular remodeling and cardiac function, ameliorated oxidative stress and inhibited apoptosis in STZ-induced diabetic hearts [377]. Together, these findings further implicate the RAAS in oxidative stress in diabetes, and suggest that ACEi and ARBs used to treat diabetic subjects may at least partially exert beneficial cardiovascular effects by attenuating oxidative stress in the heart.

10.5. Others

In addition to the aforementioned clinically used agents that may reduce cardiac oxidative stress, additional anti-diabetic or cholesterol lowering drugs have shown antioxidant properties in human trials. For example, the thiazolidinedione, rosiglitazone, reduced plasma peroxide levels in non-diabetic obese subjects [378]. Similarly, metformin restored markers of antioxidant status in patients with T2DM [379]. Lastly, reductions in cardiovascular events by statins have also been attributed to anti-inflammatory and antioxidant properties [380]. Nevertheless, the potential of these agents to prevent or reverse deleterious effects of diabetes-induced oxidative stress in the heart requires additional exploration.

11. Reductive stress in diabetic cardiomyopathy

While oxidative stress in the diabetic heart has been extensively documented, the role of reductive stress (RS), due to excess NADH, glutathione, and NADPH [381], has garnered less attention. Considerable evidence suggests that hyperglycaemia and other metabolic disturbances associated with diabetes result in the generation of ROS, ultimately leading to increased oxidative stress in a variety of tissues [382]. Glucose and fatty acid metabolism involves redox reactions that include electron extraction, storage, and transportation. Electrons are mainly stored in NADH, therefore, prolonged hyperglycemia for example could increase the NADH levels, which could tip the redox balance towards RS. The reductive power through nicotinamide adenine dinucleotides is crucial for several cellular processes. In the diabetic setting, alterations in the NAD^+/NADH ratio due to increased NADH could generate reactive oxygen species, which induce the formation of intracellular advanced glycation end products [383,384]. Of interest, changes in α-hydroxybutyrate levels in liver that were associated with insulin resistance and mitochondrial disorders induced hepatic reductive stress (RS), which contributes to metabolic traits and cardiometabolic disease [385]. Thus, aberrant NADH redox balance may play an important role in the pathogenesis of diabetes and its complications.

An imbalance of NAD^+/NADH often occurs as a result of impaired complex I function in concert with increased glucose flux via the sorbitol pathway [386] leading to ROS overproduction upon overwhelming the ETC and antioxidant defense capacity [387]. Notably, metabolites of the polyol pathway were found to be highly increased in the ventricles of diabetic rats [388]. In fact, this metabolic imbalance has been implicated in hemodynamic impairments observed in diabetic tissues [389, 390]. Particularly, aldose reductase (which utilizes NADPH in the conversion of glucose to sorbitol) is activated by glucose and is therefore thought to be involved in HG-driven complications of diabetes [391]. Importantly, inhibition of the key enzymes of the polyol pathway (aldose reductase or sorbitol dehydrogenase) protects against myocardial ischemia-reperfusion injury and prevents the decline in cardiac function in T2D rats [392]. Given that overproduction of ROS may develop as a result of NADH-imposed reductive stress, strategies to prevent reductive stress (RS) may provide novel therapeutic approaches for the treatment of DbCM.

Although the generation of ROS is a metabolic process, the impact of ROS and the extent of the resultant oxidative stress are often controlled by antioxidants that are transcriptionally regulated through Nrf2. Nrf2 depletion and downregulation of antioxidant genes results in dysfunctional myogenic tone in diabetes, which was reversed by sulforaphane, an Nrf2 activator [393]. Genetic overexpression of Nrf2 prevents the onset of T2D in mice and pharmacological activation of Nrf2 reduces oxidative stress, and a myriad of diabetic complications, including cardiovascular complications, nephropathy, and neuropathy [236,394, 395]. A recent study has shown that ChREBPα increases anabolism and mitochondrial mass through Nrf2 signaling [396]. Of note, activation of Nrf2 is essential for glucose-mediated proliferation in rodent and human β-cells [396].

Overall, these investigations indicate the positive impact of Nrf2 in mitigating diabetes and its associated complications. However, after the emergence of the “reductive stress” concept, a dual role of Nrf2, particularly if overactivated is being reported in diverse pathologies including diabetes [246,397,398]. Thus it is evident that both oxidative and reductive stress could play a significant role in the pathogenesis and progression of T2DM. Given that oxidative stress originates from NADH-imposed RS [383,399], attenuating hyperglycemia-triggered RS may provide a potential therapeutic opportunity for preventing the development of diabetes and diabetic complications.

12. Conclusions

In the current review, we present evidence supporting the role of oxidative stress as an important contributor to the development of DbCM. Defects in both ROS-producing enzymes, as well as ROS detoxification enzymes are considered to render the heart susceptible to oxidative damage leading to cardiac dysfunction. While several novel therapeutic targets have been explored in animal models of both T1D and T2D, confirming their potential in large animal models of diabetes in future studies will provide additional insight and increase the possibility of success in human trials. To date, translating antioxidant therapies into human treatment remains a major challenge, related to many factors such as dosing issues, questionable efficacy in human cardiac cells, or failure to target relevant molecular, functional and structural alterations in the diabetic heart. Thus, in

addition to a more detailed understanding of the molecular LV changes and the clinical outcomes of subjects with diabetes, significantly more clinical trials need to be performed to close the gaps between preclinical findings and clinical outcomes, particular in those with preclinical signs of DbCM. In the interim, patients with diabetes may benefit from longstanding therapies such as ACEi or ARBs, and from the recent introduction of antidiabetic drugs such as SGLT2i or GLP-1RA that may partially protect the heart by exerting antioxidant properties.

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Abbreviations

ATP	adenosine triphosphate
AMVM	adult mouse ventricular myocytes
ALX	alloxan
AS	Angeli’s salt
ACE	angiotensin-converting enzyme
ARB	angiotensin receptor blockers
ARNi	angiotensin receptor-neprilysin inhibitor
ARE	antioxidant response elements
ASOs	antisense oligonucleotides
BCL-2	B-cell lymphoma 2
BAX	BCL-2-associated X
Ca^{2+}	calcium
CAPN4	calpain small subunit 1
CM	cardiomyocyte
CV	cardiovascular
CASP	caspase
CAT	catalase

CoQ10	coenzyme Q10
CAD	coronary artery disease
DbCM	diabetic cardiomyopathy
DM	diabetes mellitus
DPP-4	dipeptidyl peptidase-4
HF	heart failure
ETC	electron transport chain
eNOS	endothelial NOS
GLP-1RAs	GLP-1 receptor agonists
GLP-1	glucagon-like peptide-1
GRX	glutaredoxins
GSH	glutathione
GSSG	glutathione disulfide
GSH-Px	glutathione peroxidase
GSR	*glutathione reductase*
GK	Goto-Kakizaki
HO	heme oxygenase
HG	high glucose
HPLC	high-performance liquid chromatography
HFrEF	HF with reduced ejection fraction
HFpEF	HF with preserved ejection fraction
H_2O_2	hydrogen peroxide
$OH^{\bullet}$	hydroxyl
iNOS	inducible NOS
IMM	inner mitochondrial membrane
IFM	interfibrillar mitochondria
ISDBB	insulin-dependent spontaneously diabetic BB Wistar rats
IL	interleukin
Keap1	Kelch-like ECH-associated protein 1

KO	knockout
LV	left ventricular
lnRNA	long-noncoding RNAs
LVDV	LV diastolic volume
MDA	malondialdehyde
MT	metallothionein
MPTP	mitochondrial permeability transition pore
mPHGPx	mitochondria phospholipid hydroperoxide glutathione peroxidase 4
mtDNA	mitochondrial DNA
MAO	monoamine oxidases
MRP1	multidrug resistance protein 1
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
NOX	NADPH oxidases
NQO	NADPH quinone dehydrogenase
NRCM	neonatal rat cardiomyocyte
nNOS	neuronal NOS
NO	nitric oxide
NOx	NO and nitrogen dioxide
NOS	NO oxidases
NRF2	nuclear factor erythroid 2-related factor 2
NAC	*N*-acetylcysteine
OHP2	oral hypoglycemic peptide 2
OLETF	Otsuka Long Evans Tokushima Fatty
OXPHOS	oxidative phosphorylation
O-GlcNAc	O-GlcNAcylation
OGA	O-GlcNAcase
OGT	O-GlcNAc-transferase

PS	peak shortening
PRX	peroxiredoxins
$ONOO^{\bullet-}$	peroxynitrite
SH	protein sulfhydryl
mPHGPx	phospholipid hydroperoxide glutathione peroxidase 4
RAAS	renin-angiotensin-aldosterone system
ROS	reactive oxygen species
SGLT2i	sodium/glucose co-transporter 2 inhibitors
STZ	streptozotocin
SSM	subsarcolemmal mitochondria
$O_2^{\bullet-}$	superoxide
SOD	superoxide dismutase
MS/MS	tandem mass spectrometry
dUTP	terminal deoxynucleotidyl transferase
TUNEL	nick end labeling
TBARS	TBA reacting substances
BH4	tetrahydrobiopterin
TBA	thiobarbituric acid
TG	transgenic
TPP^{+}	triphenylphosphonium cation
T1D	type 1 diabetes
T2D	type 2 diabetes
UPR	unfolded protein response
XO	xanthine oxidase
CDNB	1-chloro-2,4-dinitrobenzene
3-NT	3-nitrotyrosine
4-HNE	4-hydroxynonenal
OGG-1	8-oxoguanine glycosylase
15-F2t-IsoP	15-F2t-isoprostane

γ-GCS	γ-glutamylcysteine synthetase

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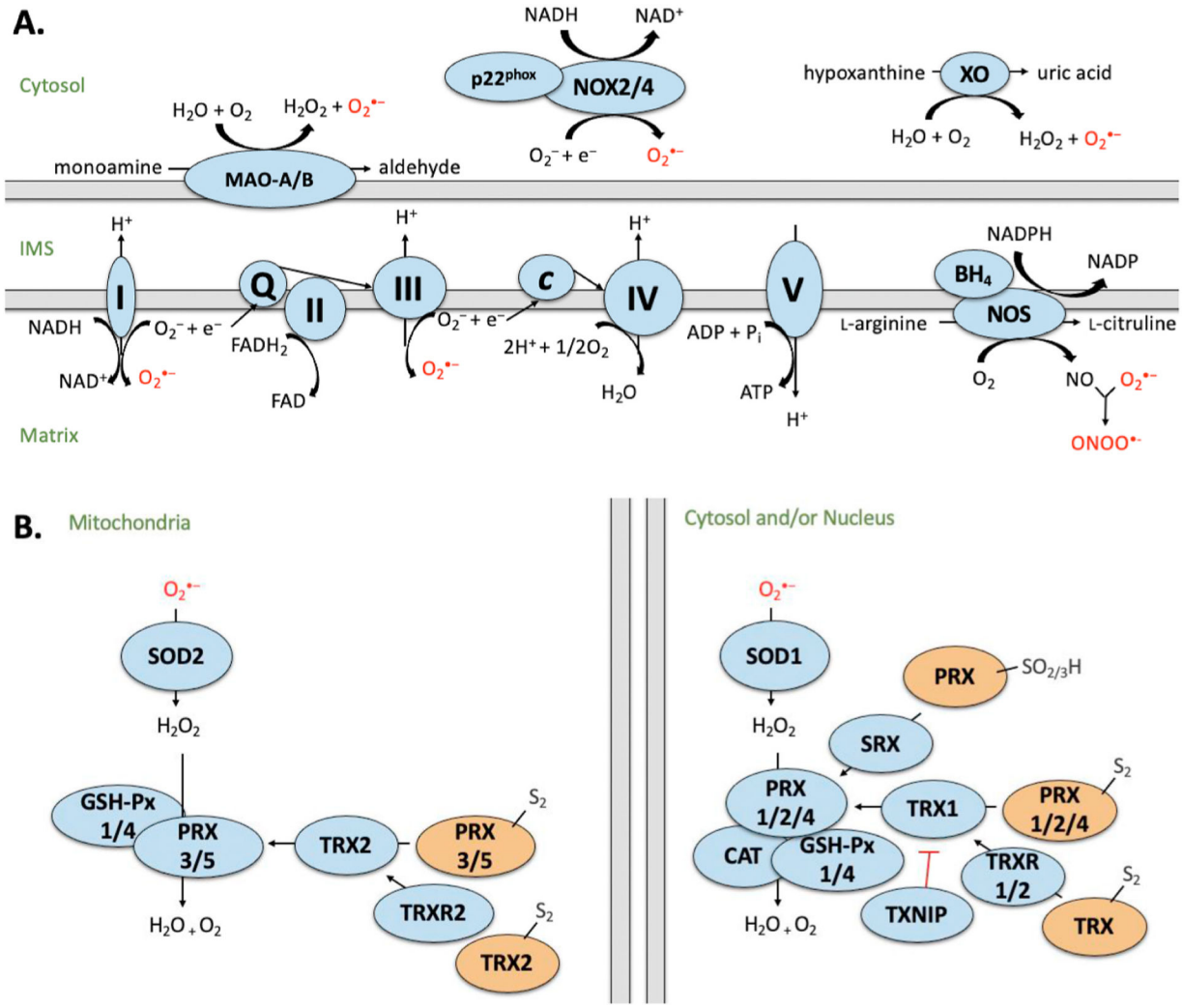

Fig. 1. Simplified schematic of A) sources of ROS and B) ROS detoxification by the antioxidant defense system.

Sources of ROS generation in the cardiomyocyte including mitochondrial electron transport chain (ETC), monoamine oxidases (MAO), calpains, nicotinamide adenine dinucleotide (NADH) oxidases (NOX), xanthine oxidase (XO) and uncoupled nitric oxide synthase (NOS). Complex I (NADH dehydrogenase) transfers electrons from NADH and passes to ubiquinone (Q). Complex II (succinate dehydrogenase; SDH) donates electrons from succinate (as part of the Krebs cycle) to Q via FeS clusters. Complex III (cytochrome *c* oxidoreductase) transfers the electrons carried by ubiquinol (QH2) to cytochrome *c* (Cyt *c*). Complex IV (cytochrome *c* oxidase) transfers electrons (e−) from cytochrome *c* to O2 to generate water (H2O). Complex V (F1F0 ATP synthase) exchanges protons from the IMS to the matrix, which drives phosphorylation of ADP to form ATP. MAO generates H_2O_2 and reactive aldehydes as by-products during the oxidation of monoamines. NOX forms a heterodimer with $p22^{phox}$ and produces $O_2^{\bullet -}$ upon electron transfer from NADPH to molecular O_2. XO reacts with molecular oxygen to produce O_2^- and H_2O_2 during the production of uric acid. NOS consumes NADPH and O_2 to convert L-arginine to L-citrulline,

generating NO and NADP as by-products. I–V: Denotes ETC complex number. **B)** Generation of ROS is counterbalanced by the antioxidant defense system. Superoxide is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 is converted to H_2O by glutathione peroxidase (GSH-Px), peroxiredoxin (PRX) or catalase (CAT). Activity of PRX is regulated by sulfiredoxin (SRX) and thioredoxin (TRX), which is further regulated by TRX reductase (TRXR) and its endogenous inhibitor, thioredoxin interacting protein (TXNIP). Blue indicates active state. Orange indicates inactive state. Intermembrane space (IMS). Proteins have been compartmentalized according to their predominant intracellular localization.

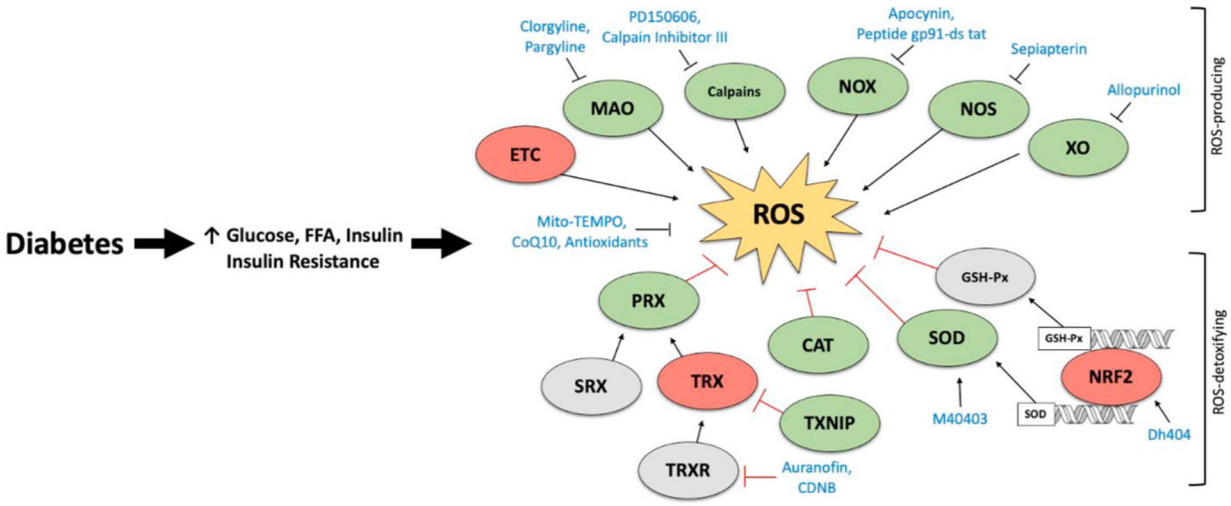

Fig. 2. Targeting ROS-producing and antioxidant enzymes in the diabetic heart.

Enhanced expression/activity of reactive oxygen specifies (ROS)-producing enzymes induces ROS overproduction in the diabetic heart. Although production of ROS is counterbalanced by enhanced expression of the antioxidant defense system, insufficient ROS-detoxification may impair the “redox state” of the diabetic heart, thereby resulting in oxidative stress. Pharmacological inhibition of ROS-producing enzymes or activation of ROS-detoxifying enzymes offers a potential therapeutic approach for the treatment of diabetic cardiomyopathy (DbCM). Green indicates elevated expression/activity in DbCM. Red indicates reduced expression/activity in DbCM. Grey indicates unchanged or unknown expression/activity in DbCM. Blue indicates pharmacological activators/inhibitors. Catalase (CAT); 1-chloro-2,4-dinitrobenzene (CDNB); electron transport chain (ETC); free fatty acid (FFA); glutathione peroxidase (GSH-Px); monoamine oxidase (MAO); nitric oxide oxidase (NOS); nicotinamide adenine dinucleotide phosphate oxidase (NOX); peroxiredoxins (PRX); sulfiredoxin (SRX); superoxide dismutase (SOD); thioredoxin interacting protein (TXNIP); thioredoxin (TRX); TRX reductase (TRXR); xanthine oxidase (XO).

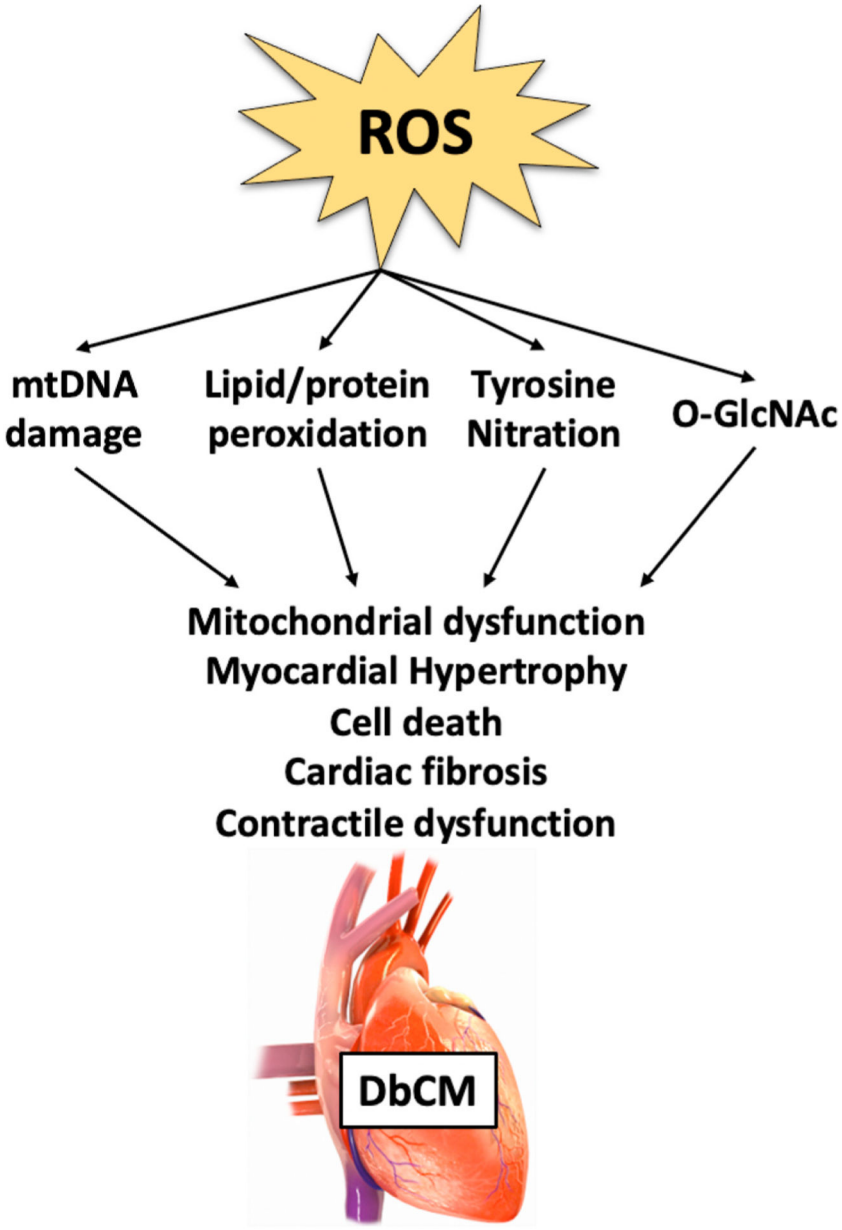

Fig. 3.

Consequences of ROS in the diabetic heart. Imbalance of reactive oxygen species (ROS) production and detoxification in the diabetic heart may result in damage to nucleic acids, proteins, and lipids, thereby resulting in mitochondrial dysfunction, myocardial hypertrophy, cell death. The convergence of these mechanisms leads to damage, which contributes to cardiac dysfunction observed in diabetic cardiomyopathy (DbCM). O-GlcNAcylation (O-GlcNAc); mitochondrial DNA (mtDNA).

Table 1

Overview of ROS, oxidative stress and cardiac structure and function in DbCM.

Diabetes Model	ROS/Oxidative Stress	Cardiac Function/Structure	Author
Permeabilized atrial myofibers from T2D patients (LVEF >30%)	↑ mitochondrial H_2O_2 emission ↓ GSH/GSSG ratio ↑HNE, 3-NT	↔ systolic function	Anderson [74]
Obese T2D patients without HF, CAD and hypertension	↑ glycogen ↑ HO-1, NQO1	↑ LV hypertrophy ↑ myocardial fibrosis	Li [234]
Right atrial myocardium from diabetic patients without signs of CM	↑ ROS levels ↑ MnSOD, CAT; ↔ Cu-ZnSOD activities	↓ mitochondrial function ↑ contractile dysfunction of atrial without signs ↓ complex I activity in obese/ diabetic individuals	Montaigne [77]
Human diabetic hearts	↓ NRF2 staining		Tan [233]
STZ-induced T1D guinea pigs	↑ ROS levels; ↔ GSH ↓ basal complex II state 4H_2O_2 emission ↔ SOD, GSH-Px, PRX, GSH, TRXR1/2, CAT, TRX1/2 ↑ oxidant-challenged H_2O_2 emission	↓ complex II and IV state 3 VO2 ↓ ADP phosphorylation rates	Tocchetti [38]
STZ-induced T1D rats	↑ O2•− ; ↓ complex I H_2O_2 production; ↑/↓ H_2O_2 production; ↑ CM ROS, TBARS, MDA, lipid peroxide, protein carbonyl, 8-iso PGF2α, HNE-adduct of SDH subunit, NT of SCOT; ↓ mitochondrial MDA ↓ GSH; ↑ GSH conjugate efflux; ↔ γ-GSC mRNA, GSR activity; ↑ GSH/GSSG ratio ↑ p22phox, NOX4 mRNA ↑/↓ SOD content; ↑ CAT; ↔ CuZn-SOD, GSH-Px activities; MnSOD, GSH-Px, PRX3 protein	↑/↓ cardiac hypertrophy ↓ systolic and diastolic function ↑ cardiac fibrosis, apoptosis ↑ mitochondrial dysfunction; ↓ mitochondrial transcription/ translation, respiration ↑ respiratory uncoupling ↓ ETC proteins; complex I, SDH, SCOT activities	Singh [37], Ghosh [35], Kanazawa [36] Kakkar [39], da Silva [40] Hamblin [41], Wohaieb [43], Herlein [44], Lashin [42], Turko [180], Turko [262], Guo [126]
STZ-induced T1D mice	↑ ROS generation in IFM, NT, MDA, 4-HNE; AGE protein; ↔ protein carbonyl ↓ GSH/GSSG ratio ↑ NOX2 mRNA ↑ OGT, OGA mRNA; O-GlcNAc	↔ cardiac hypertrophy ↓ diastolic function; contractility ↑ fibrosis ↑ DNA fragmentation ↑ Ca2+-handling dysfunction ↓ mitochondrial respiration	Dabkowski [24], Ceylan-Isik [25], De Blasio [26]
ALX-induced T1D rats	↑ intracellular ROS, MDA, protein carbonylation, lipid peroxide levels, ↑ NT of mitochondrial proteins; ↓ TBARS [51] ↓ GSH/GSSG ratio ↑ XO activity ↓ SOD, CAT, GST, GSR, GSH-Px activities	↑ CM disorganization ↓ mitochondrial membrane potential; ↑ cytosolic cytochrome *c* translocation ↑ apoptosis ↓ stimulated SCOT, complex I activities	Das [45], Genet [46], Parinandi [51], Turko [47]
OVE26 T1D mice	↓ mitochondrial GSH; ↑ GSSG; ↔ GSH content ↑ CAT activity	↑ CM morphological damage ↑ mitochondrial area, number, protein, mtDNA ↓ RCR, state 3 respiration, P/O, state 4 respiration	Shen [48], Shen [31], Liang [50]
ISDBB T1D rats	↑ CAT, GSSG reductase activities ↑ GSH content		Wohaieb [49]
Akita T1D mice	↓ mitochondrial H_2O_2 production; ↔ ROS ↔ SOD2, PRDX3 mRNA/protein	↔ O2 consumption	Bugger [52]
db/db obese/T2D mice	↑ total ROS, O2•− , ONOO•-, 4-HNE, 8OHdG, MDA ↓ GSH, GSH/GSSG ratio ↑ gp91phox, NOX1 mRNA; ↑ PRX5; ↔ SOD2 proteins ↓ OGG-1 activity	↑ cardiac remodeling ↓ systolic function ↑ apoptosis ↑ myocardial lipotoxicity ↓ complex III activity, ATP production, ATP/ADP ratio	Mariappan [56], Das [58], Pan [59], Li [234]

Diabetes Model	ROS/Oxidative Stress	Cardiac Function/Structure	Author
ob/ob obese pre-T2D mice	↓ GSH/GSSG; ↑ MDA, protein carbonyl ↑ p47phox, gp91phox protein		Li [114]
ZDF T2D rats	↑ ROS, MDA, protein carbonylation, ↑ CAT, GSH-Px, Cu/Zn-SOD, Mn-SOD ↑ GSH, GST, GSR ↑ HO-1, iNOS protein	↑ LV hypertrophy ↓ complex I, IV activities ↓ cytochrome *c* oxidase subunit 1	Raza [60], Conti [400]
fa/fa T2D rats	↑ TBARS, lipid hydroperoxides, GSH; ↔ $O_2^{\bullet-}$ formation ↑ SOD activities		Vincent [61]
HFD-fed T2D rats	↑ TBARS, lipid hydroperoxides, GSH; ↔ $O_2^{\bullet-}$ formation ↑ iNOS synthase, NO ↑ SOD activities		Vincent [61], Banerjee [62]
fructose-induced pre-T2D rats	↔ MDA, 3-NT ↑ XO activity; ↑ PRX6; ↓ SOD2 mRNA	↑/↓ ETC mRNA ↑ apoptosis	Szűcs [63], Fan [129]
HFD-fed/STZ-induced pre-T2D [64] rats, mice [234] or miniswine [65]	↑ mitochondrial ROS; total and NOX-stimulated $O_2^{\bullet-}$ production ↓ NO production; ↑ eNOS uncoupling ↑ NRF2 pathway activation	↑ diastolic and systolic dysfunction ↑ LV mass/remodeling; CM size ↑ myocardial fibrosis, apoptosis ↑ myocardial lipotoxicity	Koncsos [64], Heinonen [65], Li [234]
Goto-Kakizaki T2D rats	↑ MDA ↓ GSH, CoQ9; ↔ α-tocopherol, CoQ10, Vitamin E	↑ O_2 consumption	Santos [68]

Table 2

Studies investigating ROS-producing enzymes in DbCM.

Intervention/Model	Diabetes Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
MAO inhibitor, Clorgyline [100] or Pargyline [101]	STZ-induced T1D rats	↓ MDA, 4-HNE ↓ MAO-A activity; ↔ MAO-A protein ↓ SOD activity; UCP3 protein; ↑ GSH-Px activity; PRX-3 protein; ↔ CAT activity; PRX-5 protein	↓ contractile dysfunction, diastolic stiffness, electrical abnormalities ↓ morphological changes, fibrosis, apoptosis ↓ mitochondrial depolarization	Umbarkar [100], Deshwal [101]
CM-specific CAPN4 KO or calpastatin-TG	STZ-induced or OVE26 T1D mice	↓ ROS, H_2O_2; ↔ TAC ↓ NOX, calpain activity	↓ systolic and diastolic dysfunction ↓ cell death ↓ CM hypertrophy ↓ fibrosis, apoptosis	Li [106], Li [107], Ni [109]
NOX inhibitor, Apocynin	STZ-induced T1D mice	↓ O2•− , production, NT, NO ↔ p-eNOS (Ser1177)	↑ systolic function ↔ cardiac remodeling ↑ CM contractility	Roe [122]
NOX4 ASOs	STZ-induced T1D rats	↓ NOX4; ↔ NOX1, NOX2 protein ↓ ROS (DHE), NOX-dependent O2•− production	↓ systolic dysfunction ↓ cardiac fibrosis	Maalouf [125]
CM-specific RAC1 KO or NOX inhibitor, Apocynin	STZ-induced T1D mice	↓ O2•− , H_2O_2 production, total/mitochondrial ROS production ↓ Rac1 mRNA/protein/activity; membrane Rac1, p67phox protein; NOX activity; Rac1, gp91phox, p67phox, p47phox mRNA ↑ TRXR activity	↓ cardiac dysfunction ↓ CM hypertrophy ↓ cardiac fibrosis, apoptosis	Li [124], Shen [121]
XO inhibitor, Allopurinol	STZ-induced T1D mice/rats	↓ ROS, 3-NT, 15-f2t-isoprostane ↓ SOD activity; MnSOD, iNOS protein, XO protein/activity; ↔ eNOS protein ↔ p22phox, p40phox, p47phox and gp91phox mRNA expression ↑ NRF2 mRNA/nuclear protein; ↓ Keap1 protein/mRNA	↓ systolic & diastolic dysfunction ↓ cardiac remodeling & hypertrophy ↓ atrial CM disorder ↓ atrial & LV fibrosis, apoptosis	Yang [128], Rajesh [130], Gao [131], Luo [132]
eNOS, iNOS and nNOS KO or BH4 precursor, Sepiapterin	STZ- induced T1D mice	iNOS−/−: ↓ MDA, 4-HNE, NT protein ↓ NOx bioavailability sepiapterin: ↓ MDA, 4-HNE, NT protein ↑ BH4 and the BH4/BH2 ratio ↓ NOx bioavailability	sepiapterin: ↑ systolic function ↓ cardiac remodeling	Jo [144]

Table 3

Studies investigating the antioxidant defense system in DbCM.

Intervention/ Model	Diabetes Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
MnSOD-TG	OVE26 T1D mice genetic lipid overload-induced T2D mice	↑ cardiac SOD activity ↑ mitochondrial MnSOD protein ↑ CAT protein/activity, GSH protein ↓ cardiomyocyte ROS ↓ mitochondrial H_2O_2, 4-HNE	↑ RCR, state 3 respiration ↓ mitochondrial mass/number; ↑ mitochondrial function ↑ CM contractility ↓ mitochondrial size ↑ mitochondrial fission	Shen [31] Tsushima [89]
CM-specific CAT-TG	STZ-induced T1D mice OVE26 T1D mice	↓ ROS, H_2O_2, MPO, 3-NT of α-KGD, ATP-α and ATP-β ↓ iNOS protein; ↔ eNOS protein ↑ SIRT2 protein ↓ MDA; CM ROS	↓ systolic & diastolic cardiac dysfunction ↓ CM contractile dysfunction ↓ LV hypertrophy ↓ myocardial disorder ↓ cardiac fibrosis, apoptosis ↓ mitochondrial damage ↑ CM contractility	Cong [182], Turdi [183] Ye [29]
mPHGPx-TG	STZ-induced T1D mice	↓ H_2O_2 production, 4-HNE, MDA in IFM	↓ systolic dysfunction ↑ ATP synthase activity in IFM ↑ mitochondrial respiration ↓ mitochondrial dysfunction	Baseler [185]
GSHPx-TG	STZ-induced T1D mice	↓ TBARS, 4-HNE ↑ GSH-Px; ↔ SOD, CAT activities	↓ CM hypertrophy ↓ fibrosis ↓ apoptosis	Matsushima [186]
TRX2 activation or TRXR inhibitors, Auranofin & CDNB	STZ-induced T1D mice	↓ ROS ↓ PRX-SO2/3H; ↔ TRX1 expression/localization	↓ CM hypertrophy ↓ contractile dysfunction	Li [190]
CM-specific TXNIP KO	STZ-induced T1D mice	↔ ROS	↔ LV hypertrophy, cardiac function ↑ inotropic reserve	Myers [194]
GSH precursor, NAC	STZ-induced T1D mice	↓ ROS, H_2O_2, TAS, NO ↑ GSH content	↓ systolic & diastolic dysfunction ↓ LV hypertrophy ↓ fibrosis, apoptosis ↑ myofilament morphology	Liu [199], Fiordaliso [200], Yildirim [201]
CM-specific MT-TG	OVE26 T1D mice STZ-induced T1D mice	↓ GSSG; ↔ GSH ↓ $O_2^{\bullet -}$, ROS, 3-NT; 3-NT of ATP synthase α ↓ Trp374/Tyr135 nitration of SCOT ↑ p47(phox)	↓ myocardial injury ↓ CM contractile dysfunction; LV dysfunction/stiffness ↓ myocardial structural derangement ↓ fibrosis, necrosis ↑ ATP synthase, SCOT activity	Liang [50] Wold [113], Cai [264], Cong [213], Cong [214]
SIRT3 KO mice	STZ-induced T1D mice	↑ ROS	↑ cardiac dysfunction ↑ necrosis ↓ ATP	Song [223]
NRF2 KO	STZ-induced T1D mice HFD/STZ-induced T2D mice	↑ 8-OHdG, MDA, 3-NT, 4-HNE ↓ NRF2, NQO1 mRNA ↑ ROS production ↓ NRF2, NQO1, HO1 mRNA/protein ↓ total, nuclear NRF2 protein ↑ 8-OHdG, MDA ↓ NQO-1, HO-1, MT mRNA; MT protein	↓ cardiac dysfunction ↓ CM hypertrophy, myocardial structural damage ↓ CM contractility ↑ fibrosis, apoptosis ↑ CM hypertrophy ↓ systolic function ↑ fibrosis	He [257], He [237], Zang [301] Gu [239]
CM-specific NRF2-TG mice	STZ-induced T1D mice	↑ 4-HNE, 8-OHdG	↑ cardiac dysfunction ↑ fibrosis, apoptosis	Zang [301]
NRF2 activator, Dh404	STZ-induced T1D mice	↑ NRF2 protein ↓ 3-NT		Tan [233]

Intervention/ Model	Diabetes Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
HO-1-TG mutHO-1-TG	STZ-induced T1D mice	HO-1-TG: ↓ p47phox, GSH-PX3 mRNA) mutHO-1-TG: ↑ p47phox, GSH-Px3 mRNA	HO-1-TG: ↓ systolic dysfunction mutHO-1-TG: ↑ apoptosis	Zhao [240]
ALDH2-TG	sucrose-fed pre-T2D mice	↑ SIRT3, HO-1 protein ↓ ROS	↓ myocardial dysfunction ↑ CM contractility ↑ intracellular Ca2+ handling ↑ NAD + activity	Hu [252]

Table 4

Effects of ROS scavengers and drugs recommended for diabetic subjects on myocardial ROS and oxidative stress.

Target	Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
mito-TEMPO	STZ-induced T1D mice or db/db T2D mice	↓ mitochondrial H_2O_2 production, intracellular ROS, protein carbonyl content, TAC ↓ gp91phox, p47phox mRNA	↓ CM hypertrophy ↓ apoptosis ↓ systolic & diastolic dysfunction	Ni [306]
Resveratrol	STZ-induced T1D mice STZ/ nicotinamide-induced T1D rats *Leprdb* T2D mice fructose-induced T2D rat pre-diabetic ZDF rats	↓ 8OHdG, MDA, 15-F2t-IsoP ↓ SIRT1, Rab7 protein, acetylated/total histone 3, acetylated/total FOXO1 ↓ TBARS, GSSG/GSH ↑ SOD ↓ nitrite/nitrate ↓ O2•− production, NT ↑ nitrite/nitrate levels; eNOS, iNOS protein ↓ NOX activity; gp91phox, gp91phox mRNA/protein ↓ ROS, AGE, protein carbonyl content, NO content ↓ NOX activity; NOX1, NOX4 mRNA ↑ SOD activity, GSH level ↑ SIRT-1 mRNA/protein/activity ↓ mitochondrial H_2O_2 emission ↔ GSH/GSSG; SOD2, CAT protein	↓ cardiac dysfunction ↓ cardiac remodeling ↓ fibrosis, apoptosis ↓ apoptosis ↔ cardiac remodeling, systolic function ↓ diastolic dysfunction ↓ cardiac hypertrophy ↓ electrophysiological abnormalities ↑ NAD, NAD/NADH ratio ↓ apoptosis ↔ systolic, diastolic function ↓ cardiac fibrosis ↔ ETC subunits, ADP respiratory kinetics ↓ lipotoxicity	Wang [318] Soufi [315] Zhang [314] Bagul [317] Beaudoin [316]
Piceatannol, a natural hydroxylated analog of Resveratrol	STZ-induced T1D rats	↓ MDA, ↑ SOD activity ↑ NRF2, HO-1 mRNA	↓ systolic dysfunction ↓ fibrosis, apoptosis	Li [244]
Quercetin	high-cholesterol diet-induced T2D rats STZ-induced T1D rats	↓ 8-isoprostane, TBARS ↑ GSH/GSSG; SOD, CAT, GSH-Px activities ↑ NRF2 nuclear translocation; HO-1 mRNA ↓ 4-HNE ↑ PRX3, TRX2 protein; TRXR2 activity; ↔ PRX5 protein; ↑ SOD activity; ↔ CAT, GSH-Px activities; ↓ UCP3 protein ↑ NRF2, NRF1 mRNA	↓ diastolic dysfunction ↓ cardiac hypertrophy ↔ cardiac remodeling, systolic function ↓ CM density ↓ cardiac cholesterol content ↑ATP levels ↓ CM hypertrophy ↓ myocardial fibrosis ↑ cardiac conductivity & contractility ↓ apoptosis	Castillo [320] Arkat [181]
Taxifolin, a dihydroquercetin	STZ-induced T1D mice	↓ MDA ↑ SOD, GSH-Px, CAT activities ↓ NOX activities	↓ cardiac hypertrophy/remodeling ↓ fibrosis, apoptosis	Sun [321]
sodium selenate or omega-3/vitamin E supplementation	STZ-induced T1D rats	↔ free/total SH, nitrite	↓ cardiac dysfunction	Aydemir-Koksoy [324]
alpha-lipoic acid	STZ-induced T1D rats	↓ MDA; ↑ GSH/GSSG ↑ MnSOD activity	↓ cardiac dysfunction ↓ fibrosis	Li [325]
vitamin E	STZ-induced T1D rats	↓ 8-iso PGF2α ↓ GSSG	↓ cardiac dysfunction	Hamblin [326]
ascorbic acid, dl α-tocopherol acetate, β-	STZ-induced T1D rats	↓ MDA ↓ XO mRNA/protein; MAO-A mRNA		Kumar [401]

Target	Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
carotene, N acetyl cysteine, selenium		↓ CAT ↓ HO-1 mRNA		
coenzyme Q10	STZ-induced T1D mice	↓ 3-NT, NOX-derived O2•− production ↓ NOX2, p47phox mRNA; p22phox protein	↓ diastolic dysfunction ↓ CM hypertrophy ↓ fibrosis, apoptosis	Huynh [30], De Blasio [28]
coenzyme Q10	db/db T2D mice	↓ MDA; O2•−	↓ cardiac hypertrophy ↓ diastolic dysfunction ↓ fibrosis, apoptosis	Huynh [27]
SGLT2 inhibitor, Empagliflozin	STZ-induced T1D mice HFD-fed T2D mice ZDF obese/T2D rats db/db T2D mice KK-Ay T2D mice	↓ cytosolic & mitochondrial ROS ↓ O2•− production ↑ mtDNA content ↑ p-eNOS, NRF2, HO-1, p-p65(Ser536) protein; ↑ NRF2, HO-1 mRNA ↓ H_2O_2, 3-NT, GSH, and lipid peroxide; ↑ NO bioavailability ↓ p-eNOSser1177, eNOS monomer ↓ ROS ↑ SOD2; ↓ NOX4; ↔ NOX2 ↓ lipid hydroperoxide, MDA ↑ GSH-Px, SOD; ↓ NOX4 protein ↑ NRF2, HO-1 protein; nuclear NRF2	↓ LV hypertrophy ↓ diastolic dysfunction ↓ perivascular/interstitial fibrosis ↓ mitochondrial injury ↑ passive force ↓ LV hypertrophy ↓ systolic &diastolic dysfunction ↓ fibrosis, apoptosis	Lee [351] Sun [256] Kolijn [352] Xue [353] Li [355]
GLP-1RA, Exenatide	HFD-fed T2D mice & STZ-induced T1D mice [362]	↑ MnSOD, CAT protein	↓ systolic &diastolic dysfunction	Ding [362]
GLP-1RA, OHP2 & Semaglutide [354], or Exendin-4 [402]	HFD/STZ-induced T2D rats [354,402],	↓ ROS production, MDA ↓ p22phox, p40phox ↑ GSH-Px1, HO-1; ↔ SOD1 mRNA	↓ LV hypertrophy ↓ systolic & diastolic dysfunction ↓ fibrosis, apoptosis ↓ mitochondrial dysfunction, fission ↓ cardiac lipotoxicity ↑ cardiac ATP	Qian [354], Wu [402]
GLP-1RA, Exendin-4	KK-Ay or HFD-induced T2D mice [403]	↓ ROS, ↓ NOX4; ↔ NOX2 mRNA ↑ SOD, GSH-Px	↓ CM hypertrophy ↓ systolic & diastolic dysfunction ↓ fibrosis ↓ mitochondrial remodeling ↓ cardiac lipotoxicity	Monji [403]
DPP-4 inhibitor, Sitagliptin [364,365,369], Vildagliptin [365,366] or Linagliptin [367,371]	STZ-induced T1D rats HFD-fed T2D mice/rats ZDF obese/T2D rats	↓ MDA, NO, GSH content ↓ SOD, CAT activities ↓ TBARS, protein carbonylation, 3-NT, MDA, 4-HNE, ROS production ↑ CAT ↓ 3-NT; ↔ ROS, RNS ↓ iNOS	↓ cardiac hypertrophy ↓ CM degeneration ↓ fibrosis ↓ cardiac hypertrophy ↓ diastolic dysfunction ↑ HR variability ↓ cardiac fibrosis ↓ mitochondrial depolarization, swelling ↓ systolic & diastolic dysfunction ↓ ultrastructural abnormalities ↑ autophagy	Al-Rasheed [364] Apaijai [365], Nath [366], Aroor [367] Zhou [369], Aroor [371]
DPP-4 inhibitor, Alogliptin [368] or MK0626 [372]	ALX-induced T1D rabbits HFD-fed T2D mice	↓ MDA, 8-OHdG, mitochondrial ROS ↔ SOD activity ↔ 3-NT	↓ cardiac dysfunction ↓ fibrosis ↑ mitochondrial respiration ↑ mitochondrial biogenesis	Zhang [368] Bostick [372]

Target	Model	ROS/Oxidative Stress	Cardiac Structure/Function	Author
			↓ diastolic dysfunction ↓ fibrosis	
ARBSs, Candesartan [37], Valsartan [375], Azilsartan [376], and Olmesartan [238]; ACI-Is, Benazepril [37] and Ramipril [30]; renin inhibitor, Aliskiren [37,375];	STZ-induced T1D mice/rats db/db T2D mice obese OLETF T2D rats	↓ ROS; NT; ↑ GSH ↑ mitochondrial SOD activity; ↔ CAT activity; ↑ cytosolic NRF2, acetylated NRF2; ↓ Keap1 mRNA ↓ 8-OHdG, NT ↓ NT; ↑ GSH ↑ mitochondrial SOD; ↔ CAT activities ↑ cytosolic/acetylated NRF2; ↓ Keap1 mRNA	↓ LV hypertrophy ↓ systolic & diastolic dysfunction ↓ fibrosis ↓ apoptosis ↓ systolic & diastolic dysfunction ↓ cardiac hypertrophy ↓ fibrosis ↓ aconitase activity; ↔ complex I/II activities ↑ NAD+/NADH ratio	Singh [37], Thomas [375], Huynh [30] Sukumaran [376] Thorwald [238]
ARNi, LCZ696	STZ-induced T1D mice	↑ GSH, GSH/GSSG ratio	↓ cardiac hypertrophy ↓ cardiac dysfunction ↓ apoptosis & fibrosis	Ge [377]