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Review

Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy[☆]

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ABSTRACT

Diabetes is a recognized risk factor for cardiovascular diseases and heart failure. Diabetic cardiovascular dysfunction also underscores the development of diabetic retinopathy, nephropathy and neuropathy. Despite the broad availability of antidiabetic therapy, glycemic control still remains a major challenge in the management of diabetic patients. Hyperglycemia triggers formation of advanced glycosylation end products (AGEs), activates protein kinase C, enhances polyol pathway, glucose autooxidation, which coupled with elevated levels of free fatty acids, and leptin have been implicated in increased generation of superoxide anion by mitochondria, NADPH oxidases and xanthine oxidoreductase in diabetic vasculature and myocardium. Superoxide anion interacts with nitric oxide forming the potent toxin peroxynitrite via diffusion limited reaction, which in concert with other oxidants triggers activation of stress kinases, endoplasmic reticulum stress, mitochondrial and poly(ADP-ribose) polymerase 1-dependent cell death, dysregulates autophagy/mitophagy, inactivates key proteins involved in myocardial calcium handling/contractility and antioxidant defense, activates matrix metalloproteinases and redox-dependent pro-inflammatory transcription factors (e.g. nuclear factor kappaB) promoting inflammation, AGEs formation, eventually culminating in myocardial dysfunction, remodeling and heart failure. Understanding the complex interplay of oxidative/nitrosative stress with pro-inflammatory, metabolic and cell death pathways is critical to devise novel targeted therapies for diabetic cardiomyopathy, which will be overviewed in this brief synopsis. This article is part of a Special Issue entitled: Autophagy and protein quality control in cardiometabolic diseases.

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Abbreviations: AGEs, advanced glycosylation end products; AMPK, AMP-activated protein kinase; AT₁R receptor, angiotensin II receptor type 1; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; CB_{1/2} receptor, cannabinoid 1 and 2 receptors; eNOS, endothelial nitric oxide synthase; ER stress, endoplasmic reticulum stress; ICAM-1, intercellular adhesion molecule 1, also known as CD54; iNOS, inducible nitric oxide synthase; MAPKs, mitogen activated protein kinases; MCP-1, monocyte chemoattractant protein-1; MMPs, matrix metalloproteinases; mTOR, mammalian target of rapamycin; NADPH oxidase/NOX, nicotinamide adenine dinucleotide phosphate-oxidase; NFκB, nuclear factor kappaB; NO, nitric oxide; NOS, nitric oxide synthases; Nrf2, NFE2L2 nuclear factor, erythroid 2-like 2; PARP-1, poly(ADP)ribose polymerase 1; PARP-1, poly(ADP-ribose) polymerase 1; RAGE, receptor for advanced glycation end product; ROS/RNS, reactive oxygen and nitrogen species; SERCA2a, sarco/endoplasmic reticulum Ca²⁺-ATPase; STZ, streptozotocin; VCAM-1, vascular cell adhesion molecule 1, also known as CD106; XO, xanthine oxidase/oxidoreductase.

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1. Introduction

Incidence of metabolic diseases, especially of obesity and type II diabetes is constantly growing worldwide. Diabetes is a well-recognized risk factor for cardiovascular diseases and heart failure [1]. The term of diabetic cardiomyopathy has been introduced by Rubler et al. back in 1972 [2]. Later the Framingham Heart Study has also demonstrated that the occurrence of heart failure in male and female diabetics is twice and five times more common, respectively, compared to age-matched control subjects [3]. Similar cross-sectional studies confirmed increased risk for heart failure development in diabetics, even when corrected for other confounding variables such as coronary artery disease, hypercholesterolemia, hypertension, and obesity [4–7]. Notably, the higher occurrence of biventricular dysfunction and cardiomyopathy in diabetics is also suggestive of the fact that diabetes is an independent risk factor or cause of cardiomyopathy [7–9].

Although it is established that multiple factors may collectively contribute to the development and progression of diabetic cardiomyopathy (e.g. hyperglycemia, insulin resistance, increased fatty acid metabolism,

microcirculatory changes, sympathetic dysfunction, myocardial inflammation, remodelling and fibrosis), the exact molecular mechanisms that trigger and fuel these major pathological processes are not entirely clear (Fig. 1).

Accumulating recent evidence suggests that the complex interplay of oxidative, nitrosative and nitrative stress [10–12] with major proinflammatory, metabolic [13] and cell death pathways [14–17] play an essential role in the development of complex biochemical [18,19], mechanical, and structural alterations associated with diabetic cardiomyopathy [8,20,21], which will be overviewed in this brief synopsis.

2. Oxidative, nitrosative and nitrative stress in diabetic cardiomyopathy

Oxidative and subsequent nitrosative damage of the myocardium and vasculature have been described as major primary mechanisms leading to pathologic alterations associated with diabetic cardiovascular complications [22–29]. Factors such as hyperglycemia, glucose autooxidation, accumulation of advanced glycosylation end products (AGEs), enhanced receptor for advanced glycation end product (RAGE) and angiotensin II receptor type 1 (AT₁R) signaling, elevated levels of free fatty acids and leptin, have been reported to contribute to increased production of reactive oxygen and nitrogen species production (ROS/RNS) in diabetic vessels and myocardium [22,24], among others. In spite of the broad availability of antidiabetic therapy, glycemic control still remains a major challenge in the management of diabetic patients [30]. Inadequate glycemic control likely accounts for the high prevalence of diabetic complications, since high glucose or fluctuating glucose levels [31] can induce acute oxidative stress that might be a critical factor in the initiation of pathologic alterations eventually leading to development of diabetic cardiomyopathy [16,24]. Oxidative protein modifications play

a pivotal role in the development of diabetic myocardial dysfunction. Oxidation of proteins involved in contractility, excitation–contraction coupling, protein folding, antioxidant defence, metabolism (mainly fatty acid and glucose), and Ca²⁺ handling have been reported in diabetic hearts (see Table 1). Oxidative modification may result in potentially harmful events including dissociation of catalytic subunits of enzymes, local or global unfolding, aggregation or fragmentation. Proteins can be oxidized directly by ROS, or by products of secondary oxidation reactions formed during lipid peroxidation (e.g., malondialdehyde or 4-hydroxynonenal). Moreover, oxidative protein modifications may also occur by reactive sugars in glycation or glycoxidation reactions [32]. Depending on the amount of modified proteins and the extent of oxidation, different degradation mechanisms are activated. Mildly oxidized proteins are broken down primarily by the proteasome, while heavily oxidized proteins (potentially forming aggregates) are degraded by the endosomal–lysosomal pathway thereafter [33]. Thus, an increase in oxidative stress might produce substrates that fuel autophagic protein degradation, at least in the first stages of oxidative injury [34]. In addition, certain proteins involved in autophagosome formation and maturation (Atg4, LC3-II) require oxidative posttranslational modifications [35,36], thus, activity of these proteins are also strongly influenced by their redox status [35]. However, the accumulation of oxidized and nitrated proteins and lipids suggest abrogated autophagic processes in diabetic cardiomyopathy that may significantly contribute to myocardial dysfunction.

Several cellular and subcellular sources were described that may account for enhanced ROS production in diabetic cardiovascular system and at other sites of diabetic complications (e.g. in the retina and kidneys) (Fig. 2). This list includes nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases) [37–39], xanthine oxidase/oxidoreductase (XO) [40,41], enzymes of the arachidonic acid cascade

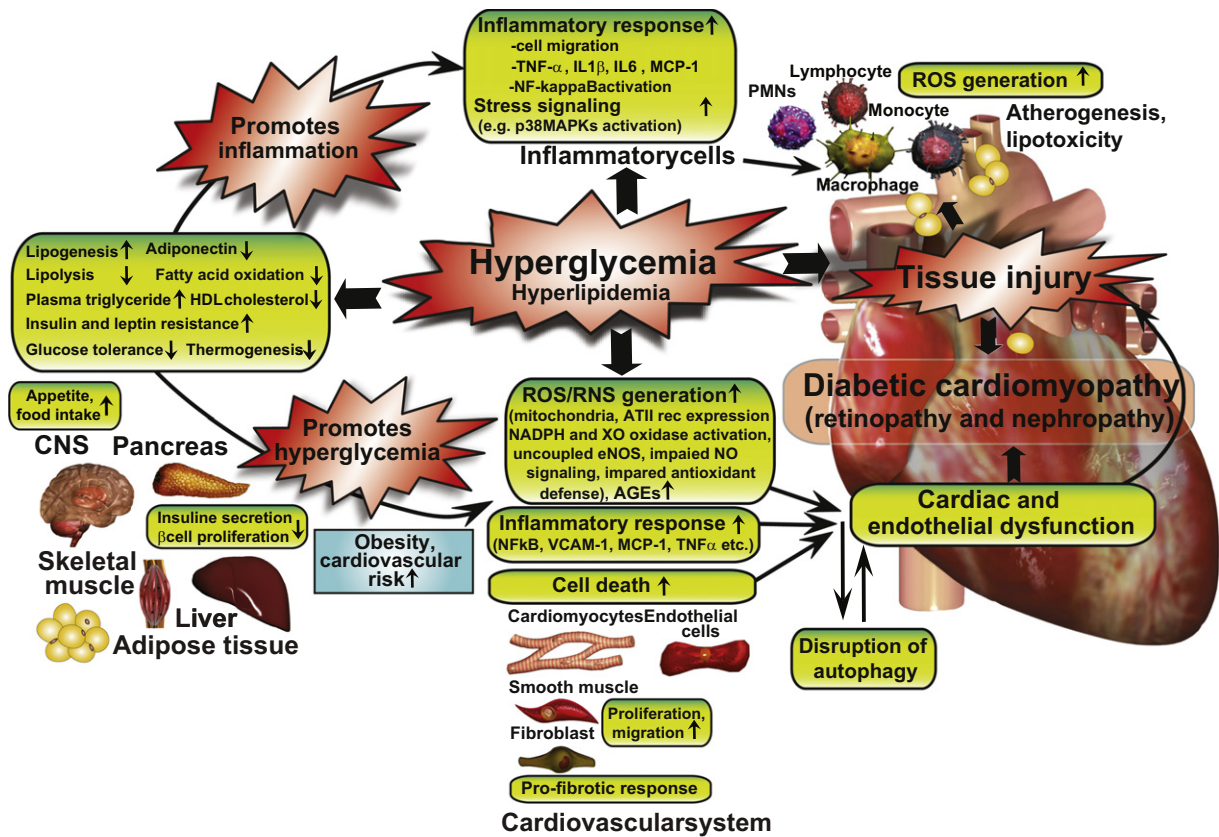


Fig. 1. Interplay of hyperglycemia and peripheral metabolism in cardiometabolic syndrome in mediating diabetic cardiovascular complications. Hyperglycemia may indirectly (via its metabolic consequences) or directly enhance diabetes-associated inflammation and ROS generation, promoting tissue injury and the development of diabetic cardiovascular and other complications. AT II rec, angiotensin II receptor type 1; CNS, central nervous system; PMNs, polymorphonuclear leukocytes; XO, xanthine oxidase.

Table 1
Oxidative and nitrate modifications of key myocardial proteins in diabetic cardiomyopathy.

Modified protein	Modification	Function	Reference
CaMK-II	Oxidation	Signaling	[45]
Protein disulfide isomerase	Oxidation	Protein folding	[140]
Complexes I, III, and V	Oxidation	ATP synthesis	[141]
mtHSP70, mitofilin	Oxidation	Mitochondrial protein import	[141]
Alpha-enolase	Oxidation/nitration	Glycolysis	[142]
Succinyl-CoA:3-oxoacid CoA transferase	Nitration	Ketone body metabolism	[143–145]
Creatine kinase	Nitration	Muscle contraction	[144]
Peroxiredoxin 3	Nitration	Antioxidant defense	[144]
Complex I (24 kDa subunit)	Nitration	ATP synthesis	[144]
Acyl coenzyme A thioester hydrolase	Oxidation	Fatty acid metabolism	[146]
Acetyl-CoA acetyl transferase	Oxidation	Fatty acid metabolism	[146]
Acyl CoA dehydrogenase	Oxidation	Fatty acid metabolism	[146]
3-ketoacyl-CoA thiolase	Oxidation	Fatty acid metabolism	[146]
Enoyl-CoA hydratase	Oxidation	Fatty acid metabolism	[146]
Heart-type fatty acid-binding protein	Oxidation	Fatty acid metabolism	[146]
Glutathione Peroxidase-1	Oxidation	Antioxidant defense	[146]
Glutathione-S-Transferase	Oxidation	Antioxidant defense	[146]
Superoxide Dismutase-2	Oxidation	Antioxidant defense	[146]
Peroxiredoxin-1	Oxidation	Antioxidant defense	[146]
Peroxiredoxin-2	Oxidation	Antioxidant defense	[146]
Peroxiredoxin-3	Oxidation	Antioxidant defense	[146]
Peroxiredoxin-6	Oxidation	Antioxidant defense	[146]
Ryanodine receptors (RyR2)	Oxidation	Excitation–contraction	[147]
SERCA2a	Oxidation	Ca ²⁺ handling	[148]
Myosin heavy chain alpha and beta	Oxidation	Contraction	[149]
GAPDH	Nitrosylation	Glycolysis	[150]
Caspase-3	Nitrosylation	Apoptosis	[150]
Connexin43	Nitration	Cell–cell communication	[151]
ATP synthase subunits	Nitration	ATP synthesis	[84]
thioredoxin-1	Nitration	Antioxidant defense	[152,153]

(cyclooxygenase and lipoxygenase enzymes), microsomal enzymes, the uncoupled nitric oxide synthases (NOS) [10], and the mitochondrial respiratory chain [24]. Among these, increased mitochondrial ROS generation initially was considered to represent the primary source of high glucose-induced oxidative stress in several tissues and cell types, including cardiomyocytes [24,37,42] and endothelial cells [43,44]. Luo et al. recently showed, that mitochondrial oxidants significantly contribute to the oxidation of the multifunctional enzyme, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) [45]. Diabetic mice treated with a mitochondria-targeted antioxidant, MitoTEMPO, showed reduced levels of oxidized-CaMKII, subsequently with preserved heart rates, and improved survival after myocardial infarction [45]. Mitoquinone, a mitochondrially targeted ubiquinone that can act as an antioxidant, also improves cardiac dysfunction [46] and activates autophagy in an Nrf2-dependent manner in a non-cardiac system [47], suggesting an interaction between mitochondrial redox balance and protein quality control mechanisms, such as autophagy.

There is also evidence for increased production of ROS from non-mitochondrial sources. NADPH oxidases (NOX) are unique enzymes that may be responsible for large amounts of superoxide and hydrogen peroxide (H₂O₂) production under various pathological conditions. Activity or expression of various NOX isoenzymes (that are involved in superoxide or H₂O₂ generation), has been found to be significantly higher in the heart with metabolic derangements such as diabetes [37–39,48,49] and hypercholesterolemia [50]. The increased activity and expression of NOX4 in the diabetic heart [49] is therapeutically targetable, and NOX4 inhibitors are indeed in preclinical development for various cardiovascular indications [51]. Diabetes induced increase in NADPH activity is further accentuated by the increased production of NADPH by glucose-6-phosphate dehydrogenase, as it was described in the heart and aorta of Zucker diabetic fatty rats [52]. Elevated concentration of hydrogen peroxide has been shown to modulate autophagy by several mechanisms. For example, H₂O₂, activates the LKB1/AMPK pathway which leads to inhibition of the mTORC1 complex and induced autophagy [53]. NOX4-mediated production of hydrogen peroxide also induces autophagy in human umbilical vein endothelial cells [54] and in

cardiomyocytes [55] after induction of endoplasmic reticulum stress. Although, it is plausible that excessive amounts of ROS might incapacitate certain players of autophagy. Vascular NADPH oxidase is also an important downstream target of angiotensin II, which has been proposed to play a pivotal pathological role in the development and progression of diabetic cardiovascular [56–59] and other diabetic complications. This is supported by convincing evidence obtained from preclinical rodent models of STZ (streptozotocin)-induced type 1 diabetes, as well as from human myocardial biopsy specimens, suggesting that the renin–angiotensin system is up-regulated in diabetes and angiotensin II locally through AT₁R, which is overexpressed in diabetic hearts or in cardiomyocytes exposed to high glucose, importantly contributes to the development of diabetic cardiomyopathy [38,57–60]. The beneficial effects of AT₁R blockade in diabetic hearts involve, but are not limited to, the attenuation of myocardial NADPH oxidase-dependent (such as p47phox) ROS generation, inflammation, cell death, fibrosis, and contractile dysfunction [57–60]. AT₁ receptor blockade also prevents glucose-induced cardiac dysfunction directly in ventricular myocytes via attenuating the AT₁-NADPH oxidase signaling [61]. Consistently with the important pathological role of NADPH oxidases-derived ROS generation in diabetic hearts, apocynin (a putative non-specific NADPH oxidase inhibitor) not only attenuated the enhanced superoxide generation in diabetic hearts, but also improved the diabetes-associated cardiac dysfunction in type I diabetic mice [39].

Increased activation of another ROS generating enzyme, the XO, has also been shown in different organs [62] of diabetic rats or mice, including in the heart [41]. Conversely, inhibition of XO by allopurinol significantly attenuated most pathological features of diabetic cardiomyopathy (e.g. myocardial ROS/RNS generation, iNOS expression, cell death, and fibrosis) and improved both systolic and diastolic dysfunctions in type I diabetic mouse [41] or rat [63] hearts. Importantly, there is also current evidence, coupled with numerous previous studies [40], for the beneficial cardiac effects of allopurinol in humans [64]. Rekhraj et al. recently described, that in patient with ischemic heart disease, high dose off allopurinol was capable to reduce left ventricular muscle mass and improve the symptoms of the disease [65].

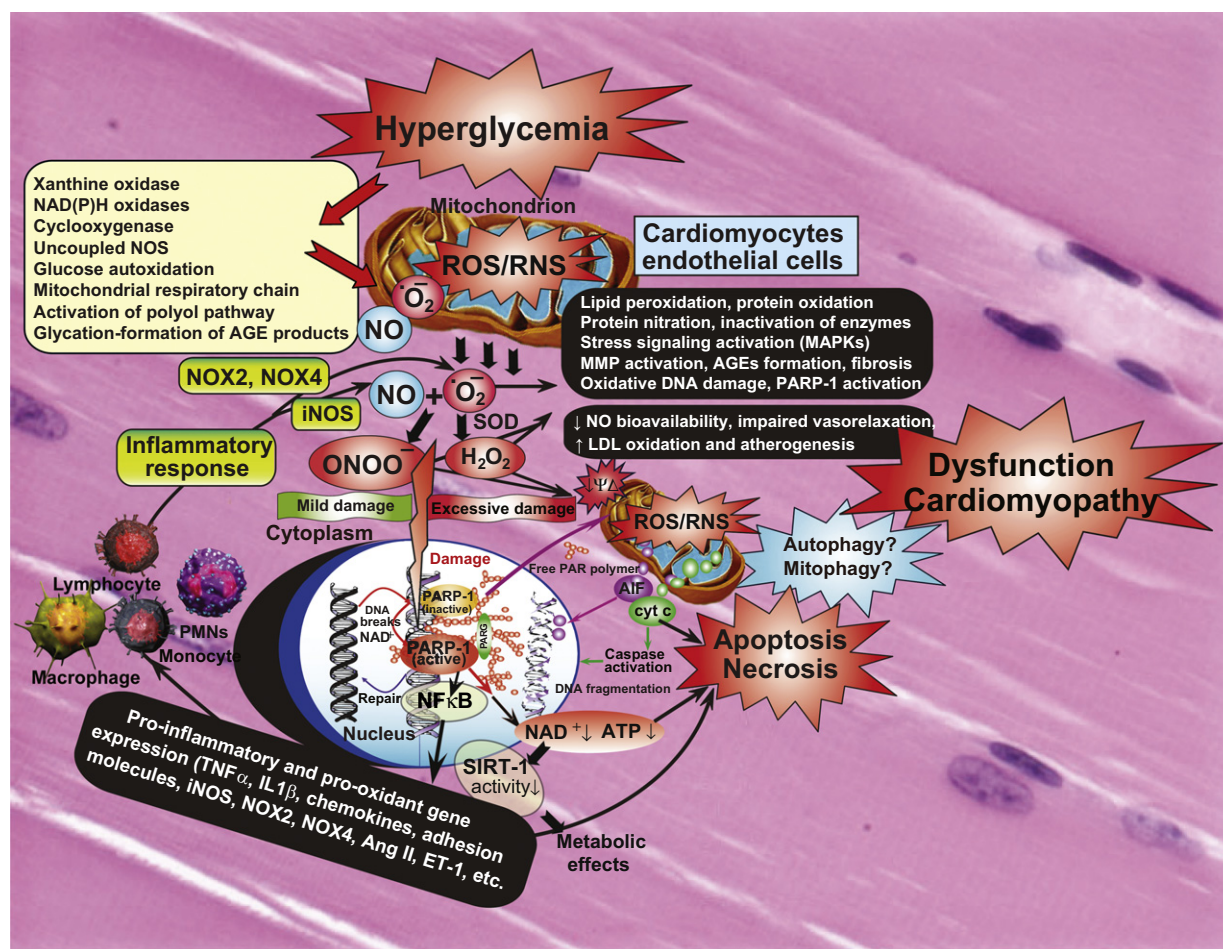


Fig. 2. Interplay of oxidative and nitrosative/nitrative stress with cell death pathways in diabetic cardiomyopathy. Hyperglycemia via activation of various pathways shown in the large yellow box increased superoxide anion ($O_2^{\bullet-}$) production in cardiovascular cell types. NO and superoxide ($O_2^{\bullet-}$) rapidly react to form peroxynitrite ($ONOO^-$) which induces cell injury via enhanced lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and also activation of stress signaling, matrix metalloproteinases (MMPs) among others. Peroxynitrite also triggers the release of proapoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria, which mediate caspase-dependent and -independent apoptotic cell demise pathways. Autophagy may be beneficial in diabetic cardiomyopathy in removal of injured cells, but additional support is required to prove its exact role. Peroxynitrite, in concert with other reactive oxidants, causes strand breaks in DNA, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Mild damage of DNA activates the DNA repair machinery, but once excessive oxidative/nitrosative stress-induced DNA damage occurs, like in diabetes, overactivated PARP initiates an energy-consuming cycle by transferring ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to nuclear proteins, resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and demise. Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose. Overactivated PARP also facilitates the activation of NF κ B and expression of a variety of pro-inflammatory genes leading to increased inflammation and associated oxidative stress, thus facilitating the progression of cardiovascular dysfunction and heart failure. Via attenuation of the cellular NAD^+ levels PARP activation may also promote metabolic dysfunction via decreased activity of SIRT-1 in various tissues. In addition to these adverse consequences the NO bioavailability and signaling is also impaired in diabetic hearts promoting impaired vasorelaxation and enhanced atherogenesis eventually facilitating increased cardiovascular inflammation, and lipid deposition in vessels and myocardium, functional ischemia and enhanced cardiac injury.

A substantial amount of experimental evidence suggests that there is reduced nitric oxide (NO) availability in diabetic tissues. Different mechanisms have been proposed to be responsible for the diabetes-induced dysfunction of NO production, bioavailability and/or signaling. A generally accepted mechanism is the alterations in nitric oxide synthases (NOS), particularly in its endothelial isoform (eNOS), function [10,66]. The composition of the eNOS complex is critical for the relative formation of NO or superoxide. The mechanisms by which eNOS dysfunction develops remain elusive, however, a decrease in the dimer to monomer eNOS ratio within the myocardium of diabetic animals has been reported [67]. Monomerization i.e. uncoupling of NOS results in increased oxidative stress and decreased NO bioavailability (since uncoupled NOS generates superoxide anion instead of NO), which has been implicated in the pathophysiology of numerous cardiovascular diseases. Accordingly, eNOS uncoupling has also been reported in diabetic hearts, while administration of the tetrahydrobiopterin precursor, sepiapterin inhibited uncoupling of NOS and improved LV function

[68]. Similarly, ascorbic acid or N-acetyl-cysteine is also capable to increase BH $_4$ /BH $_2$ ratio and prevent NOS uncoupling in the diabetic heart [69].

In addition to uncoupling the major cardiac NOS isoforms, iNOS and eNOS expression has been shown to be increased (particularly iNOS) in the diabetic hearts [37,38,70,71]. The increase in NOS expression in the diabetic heart is associated with an increase in lipid peroxidation and 3-nitrotyrosine formation (marker of peroxynitrite generation and nitrosative stress), which might be related to the uncoupled and monomer state of the enzyme, which most likely produces superoxide instead of NO, as well as triggers the formation of peroxynitrite. Interestingly, peroxynitrite itself also causes NOS un-coupling by selectively targeting and disrupting caveolae–NOS interaction [72].

The rapid diffusion-limited reaction of superoxide with NO indeed forms peroxynitrite, a very strong cytotoxic oxidant, which attacks and damages various biomolecules via multiple mechanisms [10]. In agreement with these, inhibition of NOS activity (by L-NAME

or L-NMMA) improves myocardial performance in diabetic hearts, suggesting that the increased production of superoxide and peroxynitrite rather than NO is a major contributor of suppressed contractile performance in diabetes [39,66,73,74]. NO and peroxynitrite has a very complex relation to autophagy. Nitric oxide donors have been shown to decreased autophagy in HeLa cells, which has been confirmed by the overexpression of eNOS [75]. Although, LPS-induced up-regulation of NOS isoenzymes were followed by an accelerated autophagy in HL-1 cells and in mice [76]. Since LPS induces the production of not only NO, but peroxynitrite, these seemingly opposing results can be explained. It is further evidenced by other studies, where peroxynitrite exerted inhibitory effects on autophagy. For example, peroxynitrite induced autophagy in endothelial cells and increased LC3-II protein [77]. These data well demonstrate that sub-physiological NO levels and nitrosative/nitrative stress which are characteristic to diabetes, might facilitate autophagic processes in the heart, however, direct observations are yet to be made. Peroxynitrite formation and consequent protein nitration (nitrative stress) have been suggested to play a central role in the development of cardiovascular dysfunction associated with diabetes, as well as in the development of diabetic retinopathy, nephropathy and neuropathy. Peroxynitrite in hearts not only promotes apoptotic or necrotic cell death in cardiomyocytes and endothelial cells via activation of stress signaling pathways (e.g. mitogen activated protein kinases (MAPKs) and poly ADP ribose polymerase 1 (PARP-1)-dependent pathways), but also induces nitration and consequent inactivation of key proteins involved in intracellular calcium cycling, energy homeostasis, antioxidant defense, and myocardial contractility (see Table 2.) [10]. Peroxynitrite, in concert with other oxidant may also activate matrix metalloproteinases (MMPs) in the failing hearts to promote pathological remodeling [23,37,78] and may impair the NO-soluble guanylate cyclase signaling pathway rendering NO to unable to activate its primary protective

signaling machinery [79,80]. Furthermore, peroxynitrite decomposition catalysts have been shown to restore the normal contractile phenotype of high glucose treated cardiomyocytes and attenuate cardiac dysfunction in diabetic hearts [74]. The importance of peroxynitrite has also been shown in diabetic patients. Increased serum and/or vascular 3-nitrotyrosine levels were positively correlated with increased blood pressure, and or endothelial dysfunction in diabetic patients [29,81]. The persistently increased myocardial oxidative and nitrative stress in diabetic hearts eventually also leads to dysfunction of important antioxidant defense mechanisms [10] (e.g. inactivation of important antioxidant enzymes such as superoxide dismutases and catalase, depletion of endogenous antioxidants (e.g. metallothionein, glutathione) [37, 82–84]) and dysregulation of important redox-dependent transcription factors (e.g. NFE2L2 nuclear factor, erythroid 2-like 2 (Nrf2)) [85,86], among others. Increased oxidative and nitrative stress in diabetes may also promote oxidation and/or nitration of various insulin receptors in peripheral tissues, which may contribute to the development of insulin resistance [87].

Hyperglycemia and/or hyperglycemia-induced ROS has been reported to increase accumulation of products of nonenzymatic glycation/oxidation of proteins/lipids (AGE) and enhanced the expression of their receptor (RAGE) in cardiovascular tissues, which thought to play a key role in the development and progression of cardiovascular complications of diabetes [88,89]. Accumulation of AGEs and increased expression of RAGE have been associated with diabetes-induced dysfunction and structural alterations in hearts of type 1 diabetic rodents [88,89]. Furthermore, in diabetic heart failure patients with reduced LV ejection fraction, the accumulation of AGEs and fibrosis appear to contribute to the increased diastolic stiffness, but not in patients with normal LV ejection fraction, where the increased cardiomyocyte resting tension is responsible for this phenomenon [90].

Table 2
Main therapeutic approaches and targets in diabetic cardiomyopathy.

Therapeutic target	Approach	Reference	Potential effect on autophagy
<i>Oxidative, nitrosative, nitrative stress</i>			
NADPH oxidases	<i>Enzyme inhibition</i> Apocynin, (VAS2870 or GKT137831) AT-1 receptor antagonist	[39,154] [61]	Potential inhibitors of autophagy [55,155] Potential inhibitors of autophagy [156]
Xanthine oxidase	<i>Enzyme inhibition</i> Allopurinol	[40,41,63,64]	No information
eNOS	<i>Uncoupling inhibitors</i> L-Arginine Tetrahydrobiopterin IGF-1	[157] [68] [160]	Potentially inhibits autophagy by NO [75,158] Potentially inhibits autophagy by NO [159] Potential inhibitor of autophagy [161]
iNOS	<i>Enzyme inhibition</i> L-NIL	[162,163]	No information in the literature.
Peroxynitrite	<i>Decomposition catalysts</i> MnTMPyP FP15	[28] [164]	Potential inhibitors of autophagy [77]
Mitochondria oxidative stress	MitoTEMPO	[45]	No information in the literature.
NRF2	<i>Activators</i> Sulforafan DH404	[165] [167]	Potential autophagy inducer [166]
ROCK	<i>Enzyme inhibition</i> Fasudil	[168]	Potential autophagy inducer [169]
<i>Cell death pathways—Autophagy/Mitophagy, Apoptosis, PARP-dependent cell death</i>			
p38 MAPK	<i>Enzyme inhibition</i> SB 239063	[170]	Potential inhibitors of autophagy [76]
PARP	<i>Enzyme inhibition</i> PJ34 3-aminobenzamide	[118] [171]	Potential inhibitors of autophagy [172]
AMPK activation	Metformin	[104]	Potential autophagy inducer [99]
GLP-1	GLP-1 agonists, DPP-4 inhibitors	[173–177]	Potential autophagy inducers [178]
Inflammation			
Cytokine expression (TNF α , IL1 β , TGF β)	p38 MAPK inhibition AT-1 receptor antagonist	[122] [57]	Potential inhibitors of autophagy [76] Potential inhibitors of autophagy [156]
Endocannabinoid system	CB1 antagonist	[38]	Potential inhibitors of autophagy [139]

3. Autophagy and cell death mechanisms in diabetic cardiomyopathy

It has extensively been documented that increased oxidative and nitrative stress in diabetic hearts may trigger activation of stress signalling pathways facilitating apoptosis in cardiomyocytes and endothelial cells [14]. However, recent evidence also suggests that in addition to apoptosis, other processes such as autophagy, mitophagy and PARP-dependent cell death pathways may also play an important role in controlling the cell demise during the course of cardiovascular disease [91] and diabetic cardiomyopathy [17], which will be discussed in the following part.

Autophagy is a cellular housekeeping process that is essential for removal of damaged or unwanted organelles, protein and lipid aggregates. It is a dynamic process which is tightly regulated by the availability of nutrients and the metabolic balance of the cell, and functional autophagy is indispensable for cellular survival in low-energy conditions [92]. Since in diabetes, where oxidative stress, protein- and lipid oxidation are elevated and cellular energy balance is disturbed, functional autophagy might have even higher importance in the maintenance of cardiac cellular integrity. In the heart, constitutive autophagy is a homeostatic mechanism for maintaining cardiac structure and function [93], while disruption of autophagy leads to heart failure [94]. Interestingly, in healthy animals, autophagy seems to be higher in males than in females suggesting that the male heart has a major constitutive autophagy [95]. This gender difference may elucidate the observation of the Framingham Heart Study showing markedly increased occurrence of diabetic cardiomyopathy in females [3,96]. Autophagy is suppressed at high glucose concentrations, such as present in diabetes, which might be associated with the development of diabetic cardiomyopathy [97]. In addition, the diabetic heart is characterized by excessive fatty acid uptake and oxidation, resulting in accumulation of toxic lipid intermediates (long-chain acyl-CoA, diacylglycerol, ceramides), known as lipotoxicity. Cardiac lipotoxicity contributes to insulin resistance, impairment of mitochondrial function, cardiomyocyte hypertrophy, and apoptosis, which may ultimately lead to left ventricular remodelling of the heart [98].

Elevated oxidative stress, apoptosis, inflammation, and ER stress by increased production of monocyte chemoattractant protein-1 (MCP-1) is suspected to contribute to the disruption of cardiac autophagy in diabetes [97,99–101]. Several upstream signalling pathways of autophagy are altered in diabetes or metabolic syndrome as well [102]. The mammalian target of rapamycin (mTOR) pathway, a negative regulator of autophagy is blunted in obese, hyperlipidemic rats fed a high cholesterol-high fructose diet showing most characteristics of type II diabetes [103]. Also, activity of AMP-activated protein kinase (AMPK) is reduced in high glucose conditions, leading to disturbed autophagy [104]. Although the majority of reports agree on the attenuation of the autophagy in diabetes, not all studies are equivocal (possibly due to the different experimental models used). For example, in a fructose-fed mouse model, induction of autophagy has been described [105], and milk fat-based chow blunted cardiac autophagy and facilitated cardiomyopathy more efficiently than a lard-based chow [106]. Further complicating the picture is the discrepancy that oxidative stress is elevated in diabetes, which is supposed to induce autophagy, however, the majority of studies evidence quite the opposite. This phenomenon and the lack of any direct evidence from diabetic patients warrant further investigations on the status of cardiac autophagy in disease models relevant to human pathological conditions.

Aiming to restore pathological cardiac consequences of diabetes modulation of autophagy has been attempted by different tools. Chronic activation of AMPK (therefore autophagy) by metformin or overexpression of ATG7 reduced cardiomyocyte apoptosis, cardiac fibrosis, and improved myocardial functions in streptozotocin (STZ)-induced diabetic mice [104]. Overexpression of hem oxygenase-1 in STZ-treated mice also induced autophagy, reduced oxidative stress and inflammation

and prevented cardiac dysfunction [97]. In a recent report, inhibition of mTOR signalling by the overexpression of PRAS40 prevented the development of cardiomyopathy in high-fat diet-fed mice [107]. Furthermore, it was also demonstrated that disturbed autophagy undermine the benefit of exercise in protection against high-fat-diet-induced glucose intolerance [108]. However, a few papers disagree with this conclusion. Suppression of autophagy prevented apoptosis in neonatal and adult cardiomyocytes in extremely high glucose conditions resembling to uncontrolled diabetes [109]. Furthermore, overexpression of beclin-1, an inducer of autophagy, worsened cardiac function and enhanced apoptosis in STZ-treated mice, while cardiac damage by STZ-induced diabetes was blunted in *beclin-1*^{+/-} mice [110]. In summary, although the usefulness of induction of autophagy in diabetes is still a controversial issue, the majority of reports implicate that the restoration of cellular energy household, induction of antioxidant systems and reduction of the detrimental protein synthesis by the modulation of autophagy-related pathways might serve as therapeutic targets in the treatment or prevention of diabetes and its cardiac complications, once we better understand these processes.

ROS and RNS under pathological conditions may also lead to oxidative DNA injury and overactivation of the nuclear enzyme poly (ADP-ribose) polymerase 1 (PARP-1), the predominant isoform of the PARP enzyme family, which normally participates in the regulation of DNA repair, cell death, metabolism, and inflammatory responses [111]. Following binding to damaged DNA, PARP-1 forms homodimers and catalyzes the cleavage of its substrate NAD⁺ into nicotinamide and ADP-ribose resulting in formation of longbranches of ADP-ribose polymers on target proteins such as histones and PARP-1 itself, which results in cellular energetic depletion, mitochondrial dysfunction, and ultimately necrosis. Numerous transcription factors involved in controlling inflammation such as NFκB, and various signaling molecules have also been shown to become poly(ADP-ribosylated) by PARP-1 [112]. Thus, overactivation of PARP-1 due to ROS/RNS formation not only promotes cell death by ATP and NAD⁺ depletion, but also stimulates proinflammatory mediator production. PARP inhibitors exerted marked tissue protective and antiinflammatory effects in preclinical models of ischemia-reperfusion injury, endothelial and cardiac dysfunction, circulatory shock, heart failure and diabetic complications [16,112]. More excitingly several recent studies have also suggested that PARP-1 and PARP-2 (a minor isoform of the PARP enzyme family) are involved in regulation of mitochondrial function/biogenesis, and adipogenesis in various organ systems [113,114], including in the liver, via modulation of NAD⁺ levels and consequently sirtuin 1 activity [111,115]. Specifically, PARP inhibitors in rodent models of type I diabetes were very effective in improving endothelial [116,117] and cardiac function [118], and the PARP activation from skin biopsies in microvessels positively correlated with the degree of endothelial dysfunction in prediabetic and diabetic human subjects [29]. PARP inhibition also prevented the hyperglycemia-induced pathological activation of PKC isoforms, hexosaminase pathway flux, and AGE formation in vitro, suggesting its key role in regulating pathological processes promoting the development of all major diabetic complications [16].

4. Inflammation in diabetic cardiomyopathy

Tschope et al. found using a STZ-induced rat model of type I diabetes that diabetic cardiomyopathy was characterized by significant increases in myocardial intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 (ICAM-1 and VCAM-1) expressions, as well as of beta2-leukocyte-integrins + (CD18 +, CD11a +, CD11b +) and cytokine tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β)-expressing infiltrating immune cells. These pro-inflammatory changes were positively correlated with oxidative stress and decline of left ventricular function [119]. Interestingly, transgenic activation of the kallikrein-kinin system inhibited intramyocardial inflammation, endothelial dysfunction and oxidative stress in diabetic hearts [119]. They

found that the AT-1 receptor antagonists irbesartan attenuated cardiac failure by decreasing cardiac inflammation (IL1 β , TNF α , and TGF β levels) and normalizing MMP activity, leading to attenuated cardiac fibrosis in STZ-induced diabetic cardiomyopathy [57]. Using mouse or rat models of type I diabetes they also demonstrated that neutralization of TNF α [120] or genetic deletion of kinin receptor B [121] attenuated the development of experimental diabetic cardiomyopathy associated with a reduction of intramyocardial inflammation and cardiac fibrosis. They also showed that inhibition of p38 mitogen-activated protein kinase attenuated left ventricular dysfunction by decreasing the level of myocardial pro-inflammatory cardiac cytokines in a mouse model of diabetes mellitus [122], and more recently they suggested that the STZ-induced diabetic cardiomyopathy is a robust model for investigating cardiac immune response and LV remodeling processes under diabetic conditions [123]. Several studies from other groups have also found similar pro-inflammatory changes in the myocardium of diabetic rodent hearts [37,38,41]. Recently, using type I and II models of diabetes in mice several studies have also shown activation of the major pro-inflammatory transcription factor nuclear factor kappa B in diabetic hearts [37,124] or in human cardiomyocytes or coronary artery endothelial cells exposed to high glucose concentrations which triggered enhanced ROS/RNS generation [37]. Both studies demonstrated that the

anti-inflammatory compounds used for the treatments by attenuating inflammation also decreased oxidative/nitrosative stress, myocardial cell death, remodeling, and improved diabetes-induced contractile dysfunction in rodent hearts [37,124]. On the other hand, it has been also reported that the clinically effective drugs (statins [125], RAAS inhibitors [57], metformin [126], and thiazolidinediones [127]) used in the treatment of metabolic syndrome attenuate the inflammatory response in diabetes.

Cytokines can attenuate myocyte contractility and viability by various mechanisms. For example by formation of peroxynitrite, which was shown to be a major contributor to cytokine-induced myocardial contractile failure [128]. Peroxynitrite is also a major trigger of apoptosis in cardiomyocytes exposed to various insults in vitro or in vivo [129,130]. Other proposed mechanisms may involve direct action on sarcoplasmic reticulum function and on the regulation on the SR calcium ATPase expression and activity by oxidation/nitration [10,131]. It is also known, that inflammatory cytokines may regulate the extracellular matrix composition and dynamics in the heart, which is a known important determinant of myocardial function [132]. In agreement with this, inhibition of TNF α signalling with an antibody for 6 weeks attenuated myocardial inflammation and fibrosis in experimental diabetes [120].

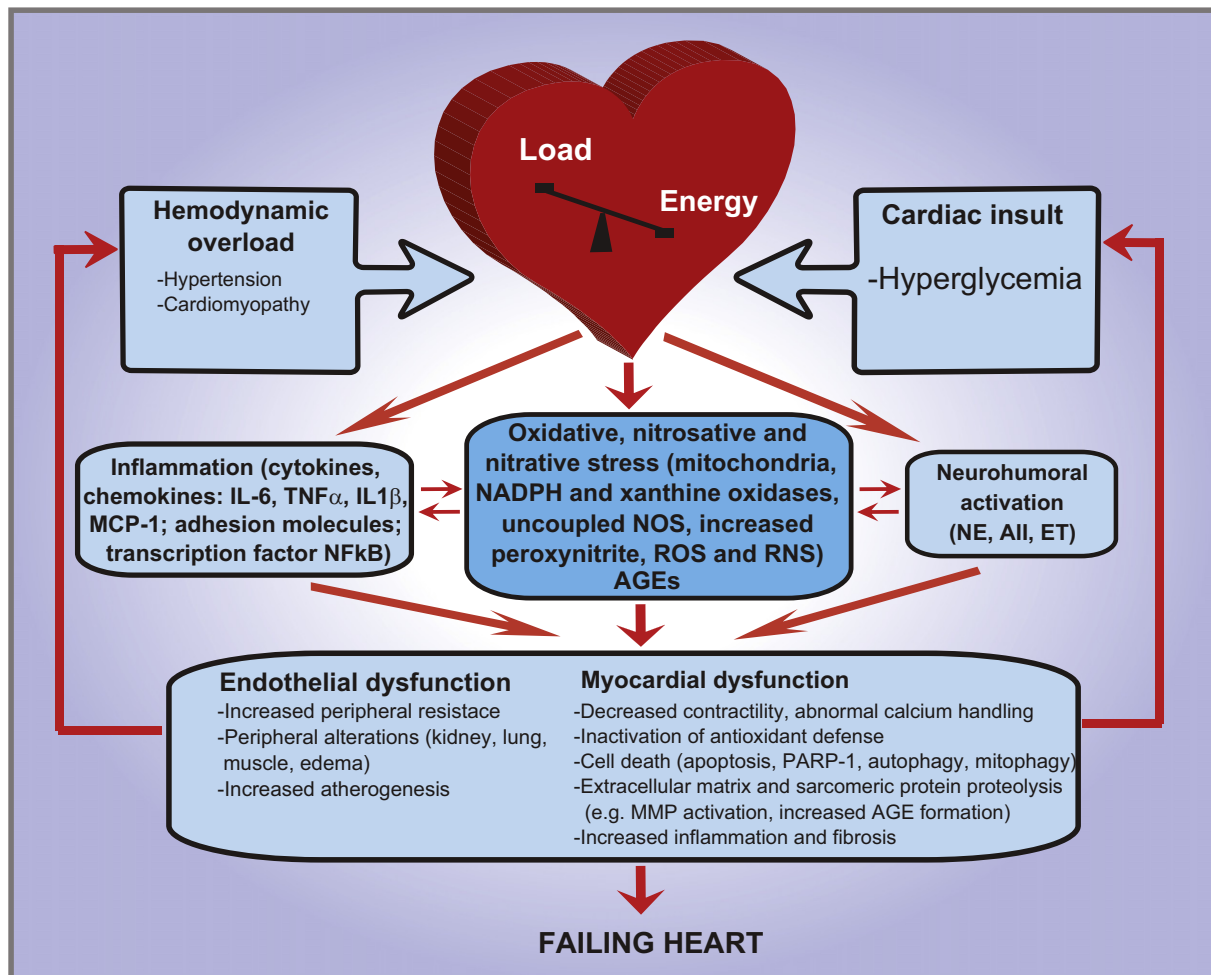


Fig. 3. Progression of diabetic cardiomyopathy: role of oxidative/nitrosative stress, inflammation, cell death and remodeling. The mechanisms leading to diabetic cardiomyopathy and failure are complex. Eventually the pathological alterations will result in mismatch between the load applied to the heart and the energy needed for contraction, leading to mechanoenergetic uncoupling. After initial insults (episodes of hyperglycemia), secondary mediators such as angiotensin II (AII), norepinephrine (NE), endothelin (ET), proinflammatory cytokines [e.g., tumor necrosis factor- α (TNF- α) and interleukin 6 and IL1 β (IL-6 and IL1 β)], in concert with oxidative stress and peroxynitrite, activate downstream effectors (e.g., PARP-1 or MMPs), act directly on the myocardium or indirectly via changes in hemodynamic loading conditions to cause endothelial and myocardial dysfunction, cardiac and vascular remodeling with hypertrophy, fibrosis, cardiac dilation, and myocardial necrosis, leading eventually to heart failure. The adverse remodeling and increased peripheral resistance further aggravate heart failure. MMPs, matrix metalloproteinases; PARP-1, poly(ADP-ribose) polymerase.

The role of myocardial cytokine signaling in the regulation of autophagy and protein quality control is unknown. In other cell types, autophagosomes are formed in response to a number of environmental stimuli, including both host- and pathogen-derived molecules, toll-like receptor ligands or various cytokines [133,134]. In particular, the cytokines IFN- γ , TNF- α , IL-1, IL-2, IL-6 and TGF- β have been shown to induce autophagy, while IGF-1, IL-4, IL-10 and IL-13 have inhibitory effect. In parallel, autophagy itself can regulate expression of several cytokines, including IL-1, IL-18, TNF- α , and IFN- γ (for an extensive review see [134]).

Recent preclinical and clinical studies have also highlighted the role of endocannabinoids (novel lipid mediators) and their G protein coupled cannabinoid receptors (CB₁ and CB₂ receptors) in the regulation of food intake, energy balance, and metabolism (for extensive review see: [135]). The endocannabinoid system also plays a prominent role in the pathology of diabetes and obesity [136,137]. We recently explored the role of endocannabinoids and CB₁ receptors in a type I model of diabetic cardiomyopathy. The diabetic cardiomyopathy was characterized by myocardial inflammation, increased expression of CB₁, increased oxidative/nitrative stress, β -MHC isoenzyme switch, AT₁R up-regulation, myocardial RAGE and AGE expression/accumulation, MAPK activation, cell death, and cardiac dysfunction (both systolic and diastolic). Pharmacological inhibition or genetic deletion of CB₁ receptors was associated with attenuated diabetes-induced myocardial inflammation, decreased MAPK activation, oxidative/nitrative stress, β -MHC isoenzyme switch, AT₁R up-regulation, myocardial RAGE and AGE expression/accumulation, MAPK activation, and largely diminished cell death and better preservation of cardiac function [38]. Recent studies indicate that cannabinoids (especially CB₁ agonists) may be important inductors of autophagy in preimplantation mouse embryos [138] and in human epithelial colorectal adenocarcinoma cells (CaCo2) [139]. Although, the evidences outlined here clearly show that inflammation is one of the key therapeutically important component associated with diabetic cardiomyopathy, to see if pharmacologic modulation of cytokine as well as cannabinoid signalling and thereby autophagy has therapeutic potential, further experimental studies are required.

5. Conclusions

Despite our accumulating knowledge based on preclinical reports, the treatment of diabetic cardiomyopathy is still largely symptomatic once it fully develops, which inevitably progresses to heart failure (see Fig. 3 for the progression of diabetic cardiomyopathy to heart failure). Therefore, an early diagnosis of this condition and rigorous glycemic control is very important to try to slow down the progression of the disease as much as possible. From preclinical and human studies it has become clear that oxidative/nitrative stress and inflammation are central components in triggering and driving the pathological processes associated with diabetic cardiomyopathy and other cardiovascular complications. However, recognizing the largely disappointing results of clinical trials with global antioxidants in diabetes, it is essential to keep in mind the necessity of more thorough understanding the complex interplay of oxidative/nitrosative stress with pro-inflammatory, metabolic and cell death signaling pathways, the specificity of these processes, and their temporal–spatial relationship.

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