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Cardiac Oxidative Stress in Diabetes: Mechanisms and Therapeutic Potential

Alyssa Faria and Shanta J Persaud

Diabetes Research Group, Division of Diabetes & Nutritional Sciences, King's College London, London SE1 1UL

Corresponding author:

Shanta Persaud

2.9N Hodgkin Building

King's College London

Guy's campus, London SE1 1UL

Tel: 020 7848 6275

Email: shanta.persaud@kcl.ac.uk

Abstract

Macrovascular complications of diabetes, including diabetic cardiovascular disease (CVD), occur through a number of hyperglycaemia-induced mechanisms that include generation of oxidative stress, accumulation of advanced glycation end-products (AGE) and activation of protein kinase C (PKC). Cardiac oxidative stress is associated with increased cardiac fibrosis and hypertrophy, and reduced cardiac performance and contractility, leading to severe cardiac dysfunction and potentially fatal cardiac events. It occurs under conditions of excessive synthesis of reactive oxygen species (ROS). The ensuing activation of transcription factors such as nuclear factor-κB produces inflammation, fibrosis, hypertrophy and further oxidative stress, which itself causes DNA and membrane damage. This review summarises the mechanisms that generate ROS in the diabetic heart: mitochondrial electron leakage, activity of ROS-generating enzymes such as NADPH oxidase, xanthine oxidase and 12/15 lipoxygenase, uncoupling of nitric oxide synthase, accumulation of AGEs and activation of PKC. There is interaction between many of these ROS-generating pathways, with data from a range of published studies indicating that a common upstream pathway is the interaction of AGEs with their receptor (RAGE), which further promotes ROS synthesis. Therefore, agents targeted at decreasing ROS production have been investigated for prevention or treatment of diabetic CVD through reducing oxidative stress, and this review considers some of the studies carried out with anti-oxidant therapies and the feasibility of this approach for protecting against diabetic cardiomyopathy.

Key Words

Diabetic cardiomyopathy, hyperglycaemia, oxidative stress, reactive oxygen species

Contents

1. Introduction

1.1 Chronic hyperglycaemia and cardiovascular disease

Diabetes mellitus is a metabolic disorder where reduced insulin sensitivity and defective insulin secretion lead to hyperglycaemia through inadequate glucose storage and inappropriate glycogenolysis and gluconeogenesis. The global burden of diabetes and the threat posed by diabetes to human health has become very severe in recent years, mainly as a consequence of increased urbanisation, population ageing and changes in lifestyle that lead to increased body mass index (BMI) [1,2]. Global projections of diabetes in 2010 proposed that 439 million adults worldwide would have diabetes by 2030[3], but already 415 million adults have diabetes and this is predicted to increase to 642 million by 2040[4].

It is the long term microvascular and macrovascular complications of chronic hyperglycaemia that lead to mortality and morbidity in diabetes. The microvascular complications affect small blood vessels and include nephropathy, neuropathy and retinopathy, while the macrovascular complications comprise cardiovascular, cerebrovascular and peripheral artery diseases[5]. The current treatments for diabetes aim to control hyperglycaemia and prevent hyperglycaemia-induced tissue damage through lifestyle changes and pharmacological interventions[6].

The increased risk of cardiovascular disease (CVD) in diabetic patients was made evident by the Framingham[7] and the MERIT[8] clinical trials, which indicated that diabetic patients have a 2 to 4-fold increased risk of CVD. Furthermore, diabetic patients have a 3-fold higher mortality rate than the non-diabetic population, highlighting that this condition is potentially fatal. Diabetic CVD encompasses a range of cardiovascular conditions, including diabetic cardiomyopathy[9], coronary heart disease and congestive heart failure[7]. Oxidative stress is believed to be involved in the pathogenesis of all of these conditions[10] and this review will focus primarily on the role of enhanced ROS production in the development of diabetic cardiomyopathy.

1.2 Diabetic cardiomyopathy

Diabetic cardiomyopathy was first described in 1972 in four diabetic patients who presented with heart failure[11]. It is a term used to distinguish between diabetes-associated cardiomyopathy, and cardiomyopathy that is associated with other co-morbidities such as hypertension or coronary artery disease[9], and these co-morbidities can significantly affect a patient's prognosis with diabetic cardiomyopathy[12]. In a study in which endomyocardial biopsies were taken from 16 diabetic patients it was determined that those subjects with symptomatic cardiac failure had the most significant myocardial changes and therefore the greatest structural and functional alterations[13.] The clinical features of diabetic cardiomyopathy include alterations in ventricular morphology such as concentric remodelling of the left ventricle leading to left ventricular hypertrophy, interstitial and perivascular fibrosis leading to reduced ventricular compliance, and diastolic dysfunction (Figure 1)[14]. These clinical

features manifest with symptoms that include shortness of breath, fatigue, weakness and ankle oedema. In addition, asymptomatic diabetic patients and individuals with pre-diabetes can also demonstrate mild changes in cardiac function, such as diastolic dysfunction[12, 15].

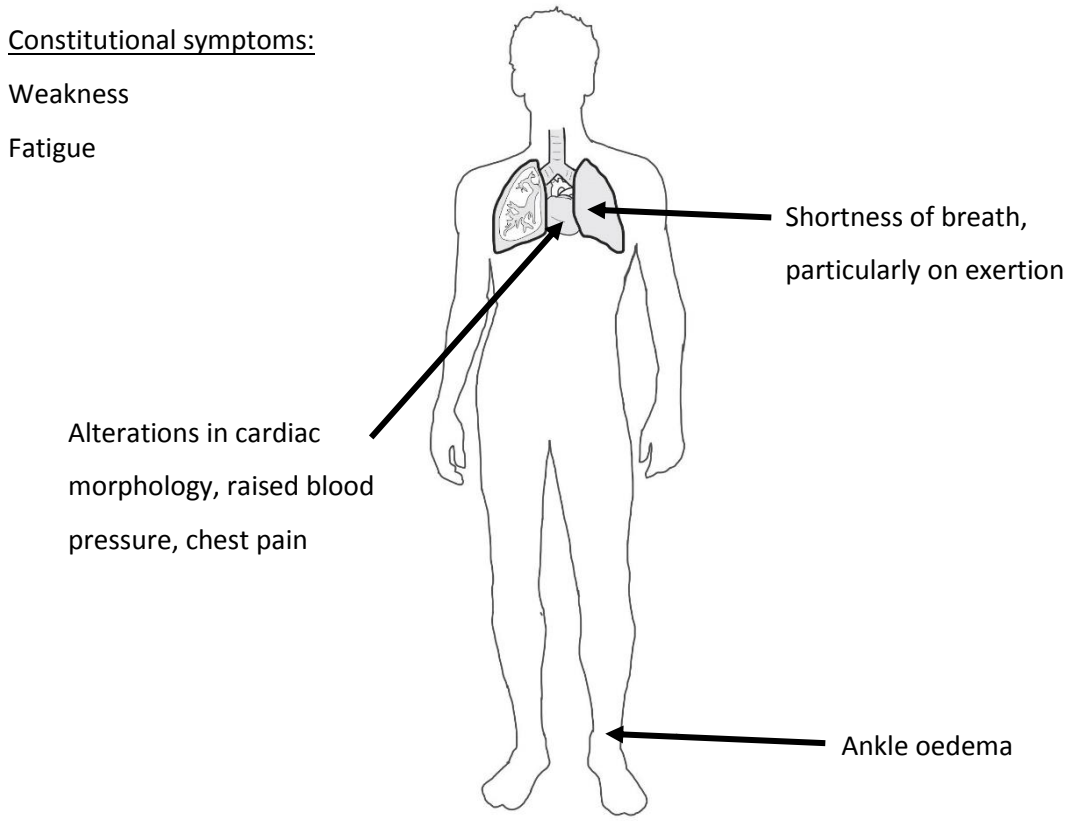

Figure 1. Clinical features and symptoms of diabetic cardiomyopathy.
The clinical features of diabetic cardiomyopathy include structural changes to the left ventricle including ventricular hypertrophy, fibrosis, reduced ventricular compliance and diastolic dysfunction. The symptoms include chest pain, elevated blood pressure, shortness of breath on exertion and ankle oedema.

1.3. Reactive oxygen species and oxidative stress

The term ROS encompasses free radical species, such as hydroxyl ($OH^{\cdot}$) and superoxide ($O_2^{\cdot}$), and non-radical species such as hydrogen peroxide (H_2O_2). Historically, ROS generation was thought to be a form of pathological cellular stress, but the current consensus is that synthesis and degradation of ROS are physiological, homeostatic functions of many cells[10,16]. However, if ROS levels are not balanced through appropriate regulation of production and removal, oxidative stress may occur and the modifications of proteins, DNA and lipids by excess ROS is associated with diabetic complications[10,16]. It has recently been reported that induction of diabetes in guinea pigs through streptozotocin (STZ) treatment led to abnormal cardiac contraction and relaxation, and isolated cardiomyocytes exhibited increased oxidative stress[17.] Furthermore, even a pre-diabetic state in rats, through administration of a single low dose of STZ, was associated with diastolic dysfunction that was accompanied by increased left ventricular mass and wall thickness [15]. Sub-sarcolemmal mitochondrial

H_2O_2 production in these pre-diabetic animals was elevated, again supporting a role for hyperglycaemia-induced cardiac dysfunction being mediated through oxidative stress.

This review summarises the pathways in diabetes that generate ROS and cause oxidative stress in the heart, illustrates the damaging effects of oxidative stress and discusses the potential role of anti-oxidant therapy as treatment for diabetic CVD, with a particular focus on diabetic cardiomyopathy.

2. Sources of ROS in the diabetic heart

In diabetes, insufficient insulin-dependent glucose uptake into fat and skeletal muscle and inappropriate glycogenolysis and gluconeogenesis leads to elevated plasma glucose levels, and this hyperglycaemia can promote elevations in ROS synthesis in the heart[18]. Figure 2 shows the pathways that generate ROS within the diabetic heart: leakage of the mitochondrial electron transport chain (ETC), increased activity of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), xanthine oxidase and 12/15 lipoxygenase (LOX) enzymes, uncoupling of nitric oxide synthase (NOS), activation of protein kinase C (PKC) and the interaction of advanced glycation end-products (AGE) with the receptor for AGE (RAGE)[10]. There may also be interactions between these pathways in the diabetic state, enabling a profound overproduction of ROS and increased oxidative stress, which can result in diabetic CVD. Specifically, the activation of RAGE in diabetes has been implicated as an upstream common pathway that promotes ROS generation through the activity of NADPH oxidase and increased mitochondrial activity.

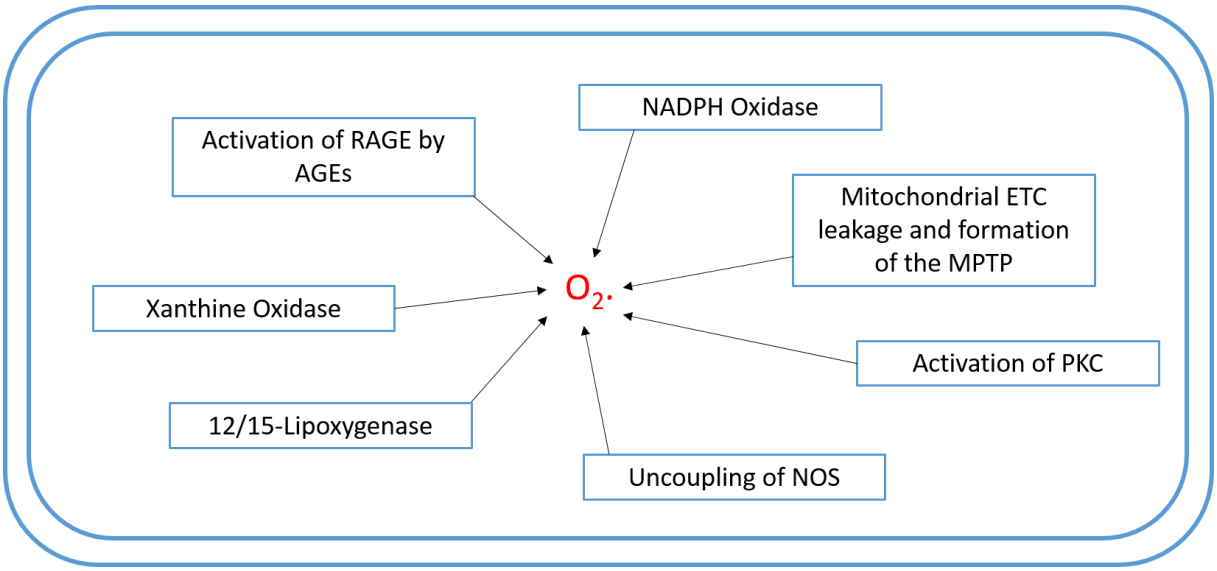

Figure 2. Superoxide ($O_2^{\cdot}$) generating pathways.
Under conditions of hyperglycaemia the following intracellular changes occur that result in increased $O_2^{\cdot}$ generation: enzymes (NADPH oxidase, xanthine oxidase, 12/15 lipoxygenase, PKC) are activated, advanced glycation end-products (AGEs) are generated, increased flux through the mitochondrial electron transport chain (ETC) causes formation of the mitochondrial permeability transition pore (MPTP), and nitric oxide synthase (NOS) is uncoupled from nitric oxide production.

2.1. The mitochondrial electron transport chain

The mitochondria are intracellular organelles responsible for oxidative metabolism of glucose to provide energy through ATP synthesis. In the hyperglycaemic state the mitochondria generate excessive $O_2^{\cdot}$ as a consequence of increased oxidative metabolic flux.

2.1.1 ROS generation via mitochondrial electron leakage

During physiological oxidative phosphorylation, there is transfer of electrons into the ETC located on the inner mitochondrial membrane. A series of complexes embedded in the membrane utilise the energy from the electrons to pump hydrogen ions (H^+) into the inter-membrane space, generating an electrochemical gradient between the inter-membrane space and the mitochondrial matrix and this is used by ATP synthase to generate ATP. The transfer of electrons into the ETC is directly related to the concentration of intracellular glucose so under conditions of hyperglycaemia the ETC will become saturated, forcing the electrons to be transferred to molecular oxygen, generating $O_2^{\cdot}$ within the mitochondria of these cells[19] (Figure 3). The $O_2^{\cdot}$ that is generated can be converted to H_2O_2 by superoxide dismutase (SOD) and then de-toxified to water by the anti-oxidant enzymes glutathione peroxidase and catalase. However, H_2O_2 can also decompose to generate highly damaging $OH^{\cdot}$ radicals and there is further insult to the ETC through the formation of the mitochondrial permeability transition pore (MPTP). Under conditions of oxidative stress, a pore opens on the inner mitochondrial membrane that allows the passage of H^+ down the electrochemical gradient into the mitochondrial matrix without ATP generation. This is known as uncoupling of the ETC and it can lead to further $O_2^{\cdot}$ generation, swelling of the mitochondrial matrix and leakage of cytochrome C, a component of the ETC, into the cytosol causing apoptosis[20] (Figure 3).

The mitochondria themselves may be susceptible to the ROS they produce, causing local damage to mitochondrial DNA and membranes, further impairing the normal activity of the ETC and therefore generating more ROS through a positive feedback mechanism[21]. Increased mitochondrial $O_2^{\cdot}$ production has been observed in a canine model of heart failure, where there was a 2-fold reduction in the activity of complex 1 of the ETC that resulted in a 2.8-fold increase in $O_2^{\cdot}$ production, as measured using electron spin resonance spectroscopy (ESR)[22]. These observations are consistent with the cardiac ETC generating ROS to impair heart function, most likely through lipid modification within the heart since there was a significant increase in 4-hydroxy-2-nonenal (4-HNE), a byproduct of ROS-induced lipid peroxidation, in myocardial sections retrieved from the dogs with heart failure.

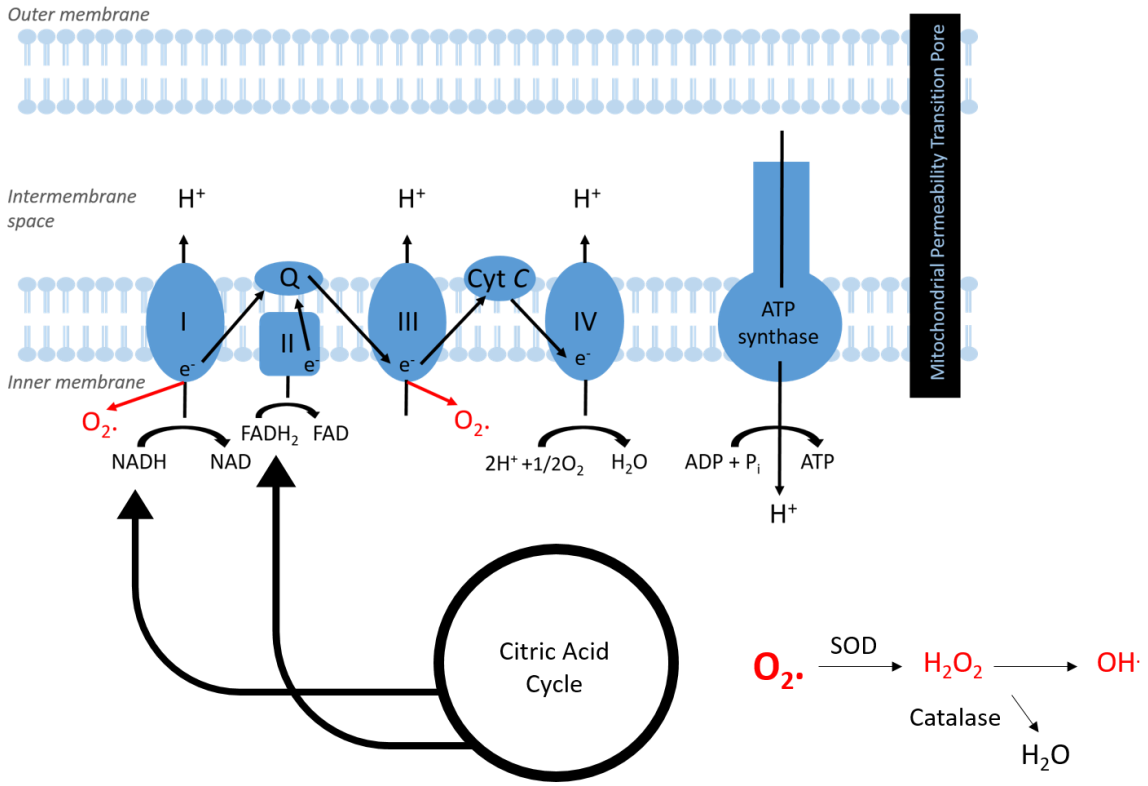

Figure 3. Superoxide production via the electron transport chain
Under conditions of hyperglycaemia increased glucose metabolism results in enhanced flux through the citric acid cycle, which increases ATP synthesis, but also leads to electron leakage that results in elevations in formation of superoxide ($O_2^{\cdot}$). The schematic shows generation of ATP and $O_2^{\cdot}$ from the electron transport chain and the position of the mitochondrial permeability transition pore.
Nicotinamide adenine dinucleotide (NAD/NADH); flavin adenine dinucleotide (FAD/$FADH_2$); electron (e^-); cytochrome *c* (cyt *c*); hydrogen ion (H^+); Complexes I, II, III and IV of the ETC (I, II, III, IV); superoxide dismutase (SOD); hydrogen peroxide (H_2O_2); hydroxyl ($OH^{\cdot}$).

Endogenously produced free radicals may not be generated in sufficiently high concentrations or they may have such short half-lives that they are undetectable by standard ESR, so spin traps can be utilised to detect free radicals. Spin traps, such as 5,5-dimethyl-pyrroline N-oxide (DMPO), bind to the free radicals and form stable adducts that can then be detected using ESR[23]. In one study in which ETC-derived $OH^{\cdot}$ radicals were measured using ESR and spin trapping with DMPO in cardiac sub-mitochondrial particles it was found that administration of SOD and antimycin, an inhibitor of the ETC which collapses the H^+ gradient, produced more $OH^{\cdot}$ adducts, thus providing further evidence for the role of the ETC in ROS generation in cardiac pathology[24]. Further experimentation showed a 2.9-fold increase in the formation of 8-hydroxydeoxyguanosine (8-OH-dG), an oxidised form of the deoxyguanosine DNA nucleoside, on addition of the citric acid cycle intermediate succinate, and a 3.4-fold increase of 8-OH-dG on addition of succinate and antimycin[24]. These results suggest that

mitochondrial DNA was oxidised by the $OH^{\cdot}$ generated, but there was no evidence of a causal relationship in this study.

2.1.2 ROS generation via alterations in the mitochondrial hydrogen ion electrochemical gradient

Antimycin mediates uncoupling of the ETC via dissipation of the H^+ electrochemical gradient and this has been associated with an increase in ROS synthesis[24]. However, other studies have shown that administration of uncoupling agents that collapse the H^+ gradient to aortic endothelial cells, under hyperglycaemic conditions, caused a reduction in ROS generation to levels not statistically different from those produced by these cells when incubated with a physiological glucose concentration (5mM glucose)[18]. A recent review provided some insight into the discrepancy observed between these two studies. It has been suggested that mild uncoupling of the ETC leads to a reduction in ETC-mediated ROS accumulation by reducing the driving force of H^+ from the intermembrane space into the matrix of the mitochondria. However, profound uncoupling of the ETC will cause full dissipation of the electrochemical gradient and generate more ROS to produce oxidative stress[25]. Therefore, the inconsistency between these studies may reflect differences in the extent of mitochondrial ETC uncoupling[18,24]. Furthermore, disparities may be attributed to differences in experimental protocol. For example, one study used intact healthy bovine aortic endothelial cells in the hyperglycaemic state[18] whereas the other used bovine sub-mitochondrial particles under normoglycaemic conditions[24]. Nevertheless, these studies provide further support for the role of mitochondria in the generation of oxidative stress, placing emphasis on the involvement of the H^+ electrochemical gradient.

2.2. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases

NADPH oxidases, phagocytic ROS-generating enzymes, are comprised of cytochrome b558, which is a membrane spanning component, and cytosolic components (Figure 4). Cytochrome b558 is composed of the phagocytic oxidases p22phox and gp91phox, and the cytosolic components comprise rac, p47phox, p67phox and p40phox[10]. NADPH oxidases have a physiological function to generate ROS as a means of cellular protection from pathogens. Individuals with chronic granulomatous disease have mutations in the phox proteins that constitute NADPH oxidase and they characteristically suffer from recurrent fungal and bacterial infections as well as inflammatory disorders such as inflammatory bowel disease, highlighting the protective role of NADPH oxidases in host defence[26].

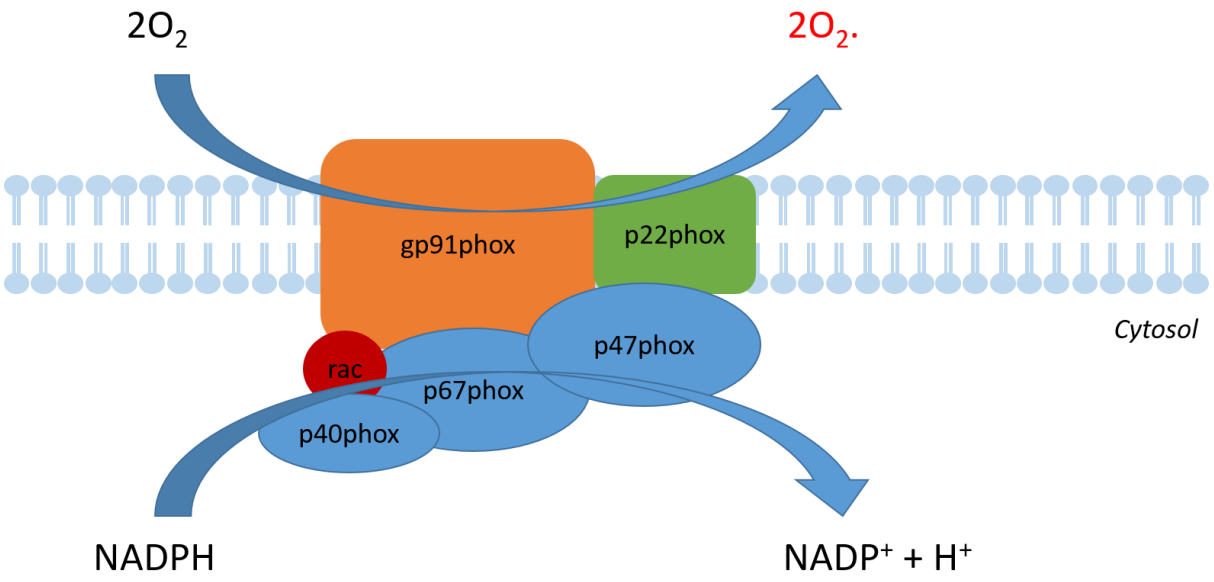

Figure 4. Components of NADPH oxidase
NADPH oxidases are enzymes consisting of multiple phox (phagocytic oxidase) subunits. Transfer of electrons from NADPH to oxygen generates superoxide ($O_2^{\cdot}$), to provide cellular protection against pathogens, and $NADP^+$ and H^+ are also produced in the process. Diabetes is associated with increased cardiac NADPH oxidase-dependent $O_2^{\cdot}$ generation, leading to the production of oxidative stress.

2.2.1 NADPH-dependent superoxide production

The cytosolic components of NADPH oxidase interact with cytochrome b558 at the membrane to produce an activated form of NADPH oxidase that catalyses the transfer of electrons to molecular oxygen, generating $O_2^{\cdot}$[10] (Figure 4). NADPH oxidase-dependent $O_2^{\cdot}$ production is reported to be significantly elevated in guinea pig left ventricular hypertrophy (LVH) tissue homogenates, and there was also an increase in expression of the NADPH oxidase component gp91phox, also known as NOX2, in the cardiomyocytes of the LVH guinea pigs[27]. This indicates that NOX2 is upregulated in the myocardium of the LVH guinea pigs, which may account for the rise in NADPH oxidase-dependent $O_2^{\cdot}$ production. NOX2 has also been implicated in the induction of cardiac hypertrophy by the vasoconstrictive peptide angiotensin II through observations that small interference RNA (siRNA)-induced knock down of NOX2 expression in cardiomyocytes abolished both angiotensin II-induced $O_2^{\cdot}$ synthesis and cardiomyocyte hypertrophy[28]. Other specific NADPH oxidase isoforms, with various cytochrome subunits (NOX), have been further investigated. NOX1 and NOX3 are not expressed by mouse heart[29] but studies have been performed to identify the role of the NOX4 isoform that is mainly expressed in the mitochondria of cardiomyocytes. Cardiac-specific NOX4 knockout mice (c-$Nox4^{-/-}$) showed a 40% reduction in $O_2^{\cdot}$ synthesis in response to pressure overload, indicating that NOX4 is important for $O_2^{\cdot}$ production by the myocardium[29]. Conversely, cardiac-specific NOX4 over-expression was associated with more pronounced effects of pressure overload, with increased cardiac dysfunction

and elevated apoptosis of cardiac cells, supporting a role for this $O_2^{\cdot}$-generating enzyme in oxidative stress in the failing heart[29].

The role of rac1, a cytosolic component of many NADPH oxidase isoforms (Figure 4), in the pathogenesis of diabetic cardiomyopathy has been investigated using rac1 knockout mice. It was found that hyperglycaemia-induced up-regulation of NADPH oxidase activity, ROS production, cardiomyocyte apoptosis and myocardial dysfunction were all significantly reduced following cardiomyocyte-specific rac1 deletion[30]. In addition, treatment of hyperglycaemic db/db mice with a rac1 inhibitor inhibited NADPH oxidase activity, as expected, and also significantly reduced cardiomyocyte apoptosis[30]. Collectively, these data are consistent with rac1 playing a central role in NADPH oxidase-dependent ROS production that contributes to myocardial dysfunction in diabetes.

2.2.2 Benefits of exercise training on NADPH oxidase-dependent oxidative stress

Studies have been designed to determine whether exercise training affects the expression of NADPH oxidase regulatory subunits, and whether this modifies myocardial redox status. For example, induction of diabetes in rats using STZ treatment led to significantly increased left ventricular mRNA and protein expression of p47phox and p67phox[31]. The higher levels of these NADPH oxidase subunits in the left ventricle of the diabetic rats were significantly reduced when the rats underwent a three week treadmill endurance exercise protocol, such that expression was similar to that of non-diabetic rats undergoing the same exercise protocol. The findings of this study suggest a mechanism by which exercise training produces improved indices of cardiac function in diabetes, which have been reported previously[32] and if these studies in rats are translatable to humans they provide support to the use of exercise training in diabetes to improve patient outcomes.

2.3. Xanthine oxidase

Xanthine oxidase, found in the cytosol of cardiomyocytes, produces H_2O_2 and $O_2^{\cdot}$ as by-products of the metabolism of hypoxanthine and xanthine into uric acid[10]. The role of this enzyme in the pathogenesis of diabetic CVD is not well established, but it is important in the pathogenesis of non-diabetic cardiac pathologies[33]. Xanthine oxidase inhibitors have been used in both animal and human studies to develop a great understanding of the role of this enzyme in cardiovascular dysfunction, as summarised below.

2.3.1. Animal studies with xanthine oxidase inhibitors

The effect of the xanthine oxidase inhibitor allopurinol on cardiovascular dysfunction has been investigated using dogs in which dilated cardiomyopathy was induced, which led to a 4-fold increase in xanthine oxidase mRNA[34]. Allopurinol treatment resulted in significantly improved myocardial contractility and performance by augmenting the increased afterload on the heart, preventing the

reductions in cardiac contractility that are observed in heart failure[34]. In humans, cardiac uric acid accumulation, prior to its excretion in urine, may contribute to the pathophysiology of cardiomyopathy but dogs possess the enzyme urate oxidase that breaks down uric acid into allantoin. Therefore, it is clear that results of studies performed with mammalian models are not necessarily translatable to the human situation, and such data should be interpreted with caution.

2.3.2. Human studies with xanthine oxidase inhibitors

Elevated uric acid has been demonstrated as a biomarker for mortality and requirement for cardiac transplantation in heart failure patients[35], so inhibition of xanthine oxidase may have beneficial outcomes in these individuals. A randomised double-blind placebo controlled trial using oxypurinol, the active metabolite of allopurinol, was conducted on patients with moderate to severe heart failure and it was found that only those patients with elevated baseline serum uric acid showed improvement with oxypurinol[36]. This suggests that inhibition of uric acid production has therapeutic potential in this subset of dilated cardiomyopathy patients, but further investigation is warranted to ascertain the precise mechanism(s) of action and therapeutic benefit of xanthine oxidase inhibitors in humans.

2.4. 12/15 Lipoxygenase

Lipoxygenases (LOX) are a family of enzymes that oxidatively metabolise arachidonic acid, a polyunsaturated fatty acid released from the plasma membrane following hydrolysis of phosphatidylcholine, into a variety of hydroperoxides. 12-LOX and 15-LOX convert arachidonic acid into 12- and 15-hydroxyeicosatetraenoic acids, releasing ROS in the process. Hyperglycaemia- induced activation of 12/15-LOX is associated with increased cardiac oxidative stress and diabetic cardiomyopathy[10].

The effects of 12/15-LOX on diabetic cardiomyopathy have been investigated using transgenic mice in which these enzymes have been deleted (12/15-LOX-KO) and which have been rendered diabetic through STZ treatment[37]. Immunofluorescence staining 4 weeks after the induction of diabetes illustrated upregulation of 12/15-LOX in the hearts of wild-type mice and these mice exhibited cardiac fibrosis. However, diabetic 12/15-LOX-KO mice exhibited reduced cardiac fibrosis than the wild-type mice, suggesting that disruption of the normal activity of 12/15-LOX is cardioprotective. In addition, levels of the lipid peroxidation by-product 4-HNE were significantly elevated in the myocardium of wild-type mice after STZ treatment but not in diabetic 12/15-LOX-KO mice[37]. These experiments therefore provide further supporting evidence of 12/15-LOX-derived oxidative stress in diabetic cardiomyopathy.

Inhibition of 12-LOX has shown promising effects in preventing inflammation of human islets of Langerhans, thereby reducing inflammatory cytokine-induced beta-cell dysfunction and preserving glucose-stimulated insulin secretion[38]. This study suggests that 12-LOX inhibition may have therapeutic

potential to preserve functional mass of human islets but, as yet, no studies have investigated the effect of inhibition of this enzyme on diabetic CVD.

2.5. Uncoupling of nitric oxide synthase

The cardiovascular system synthesises NO through the activity of nitric oxide synthase (NOS), which exists in three isoforms: inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS). Endothelial function in the cardiovascular system depends on the coupling of eNOS using the co-factor tetrahydrobiopterin (BH4). Physiological coupling of eNOS refers to the interaction of the eNOS haem group with L-arginine, the eNOS substrate, aided by BH4 during NO synthesis. BH4 is synthesised by GTP-cyclohydrolase-I (GTPCH) from guanosine triphosphate (GTP) *de novo*. Under oxidative stress, BH4 will be converted to 7,8-dihydrobiopterin (BH2), which can bind to eNOS, promote uncoupling and cause the synthesis of $O_2^{\cdot}$ instead of NO[39] (Figure 5). This therefore leads to oxidative stress.

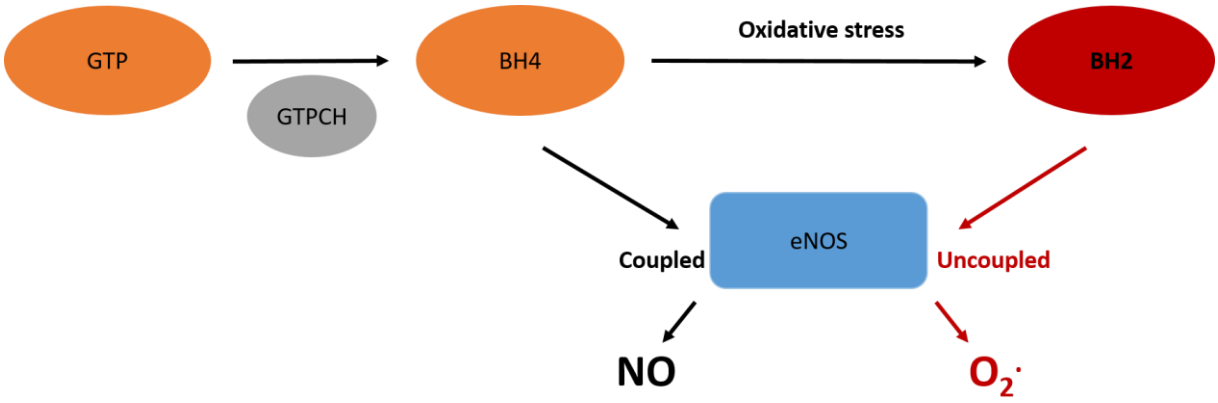

Figure 5. Uncoupling of endothelial nitric oxide synthase
Physiological coupling of endothelial nitric oxide synthase (eNOS) requires its interaction with the cofactor tetrahydrobiopterin (BH4) to generate nitric oxide (NO). Under physiological conditions, BH4 is synthesised *de novo* by GTP-cyclohydrolase-I (GTPCH) from GTP. However, under oxidative stress, uncoupling of eNOS occurs: BH4 is converted to 7,8-dihydrobiopterin (BH2), which interacts with eNOS leading to the generation of superoxide ($O_2^{\cdot}$) rather than NO, and this exacerbates the oxidative stress.

2.5.1. The role of eNOS and iNOS in ROS generation

It has been shown experimentally that up-regulation of eNOS protects against cardiac dysfunction. Thus, transgenic mice with a 30-fold increase in cardiomyocyte eNOS activity had improved LV function and reduced LVH following coronary artery ligation[40]. In contrast, iNOS may be involved in the pathogenesis of heart failure since cardiomyocyte-specific iNOS knockout mice were shown to have significantly increased survival following heart failure induced through myocardial infarction[41]. These mice also had reduced LV remodelling and demonstrated cardiomyocyte apoptosis. It has been reported that iNOS is up-regulated in the hearts of diabetic mice, which also had increased levels of 4-HNE and nitrotyrosine, a reactive nitrogen species[42]. A direct role for iNOS in the increased

stress markers was demonstrated by attenuation of 4-HNE and nitrotyrosine following knockout of the iNOS gene[42]. These findings provide further evidence for the harmful, oxidative stress-inducing role of iNOS in diabetes. However, studies have also shown that a 40-fold increase in iNOS activity alone did not induce heart failure, indicating that increased iNOS activity *per se* does not trigger cardiac dysfunction[43].

2.5.2. The effect of increasing BH4 levels on ROS generation

The importance of the BH4 synthesising enzyme GTPCH in normal cardiac function has been demonstrated in a recent study in which 4 weeks' administration of 2,4-diamino-6-hydroxy-pyridine (DAHP), a GTPCH inhibitor, to C57BL/6 mice led to significant reduction in cardiac GTPCH activity that was accompanied by impaired function[44]. Of particular interest was the observation that transgenic mice overexpressing human GTPCH in their cardiomyocytes showed significantly attenuated diabetes-induced cardiac remodelling and they had improved calcium handling[44]. While it is not possible, and not necessarily desirable, to overexpress GTPCH in humans, the observations of the association between reduced cardiac GTPCH activity and cardiac dysfunction suggest that maintaining activity of this enzyme in the heart may be a therapeutic option to improve cardiac function in diabetes. However, identifying the factors responsible for reductions in GTPCH activity in diabetes and selectively preventing this from occurring in the heart is unlikely to be therapeutically feasible, so a more practicable approach may be through elevating BH4 levels to compensate for the reductions in GTPCH.

The efficacy of sepiapterin, a BH4 precursor downstream of GTPCH, on the generation of NOS-derived oxidative stress in the diabetic mouse heart has been examined[42]. It was found that sepiapterin administration to diabetic mice significantly increased the concentration of cardiac BH4, inhibited production of stress markers stress such as 4-HNE and nitrotyrosine and improved cardiac performance. However, the iNOS knockout mice did not respond favourably to sepiapterin treatment, indicating that physiological coupling of iNOS using BH4, to promote NO synthesis and reduce ROS generation, is cardioprotective in diabetes[42]. These findings may explain why an earlier study did not observe heart failure in transgenic mice with increased iNOS activity[43]: it appears that it is enhanced generation of ROS via the uncoupling of iNOS that promotes cardiac dysfunction, rather than simply an increased expression or activation of the enzyme. A synthetic form of BH4 is well tolerated for treatment of the rare genetic condition phenylketonuria[45], so BH4 replacement therapy may be possible for reducing diabetes-induced cardiovascular complications by promoting appropriate coupling of NOS enzymes and reducing oxidative stress.

2.6. Signalling via the receptor for advanced glycation end-products (RAGE)

Chronic hyperglycaemia leads to non-enzymatic covalent bonding of carbohydrates, such as glucose, to proteins and lipids in a process known as glycation. Glycation products that form in the

short-term can combine to form cross-linked structures known as advanced glycation end-products (AGEs). These modified proteins and lipids can bind to a cell surface receptor for AGE (RAGE) that is present on macrophages and endothelial cells and trigger a cascade of events through which ROS generation and activation of nuclear factor (NF)-κB leads to the production of pro-inflammatory cytokines (Figure 6). There is a vicious cycle in AGE signalling since NF-κB also up-regulates RAGE expression, leading to further ROS and cytokine synthesis. AGE/RAGE signalling is involved in the pathogenesis of both microvascular and macrovascular diabetic complications through the generation of oxidative stress.

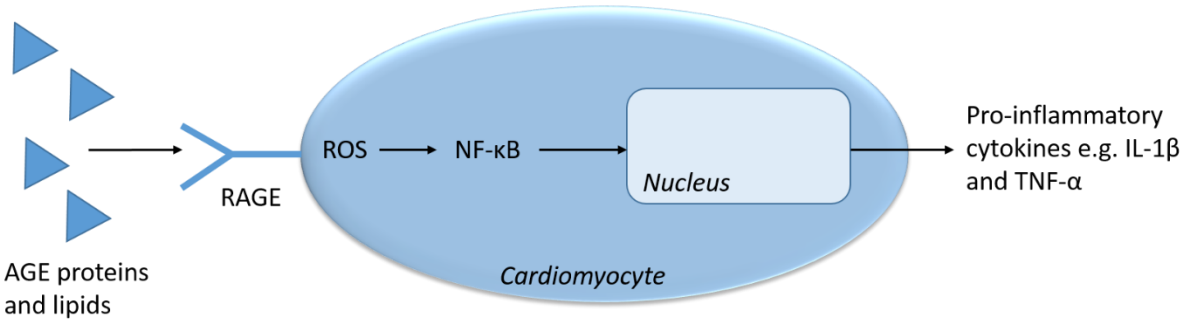

Figure 6. Interaction of AGE proteins with RAGE
Under conditions of hyperglycaemia enhanced glycation leads to the formation of cross-linked AGE proteins and lipids that bind to receptors (RAGE) to generate ROS, activate NF-κB and produce pro-inflammatory cytokines such as IL-1β and TNF-α.
Advanced glycation end-products (AGE); receptor for advanced glycation end-products (RAGE); reactive oxygen species (ROS); nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); interleukin-1β (IL-1β); tumour necrosis factor alpha (TNF-α).

2.6.1. The role of RAGE in ROS generation

It has been reported that concentrations of serum AGEs increase in healthy individuals with age, and these were associated with elevated levels of oxidative stress and inflammation[46]. Since chronic hyperglycaemia in uncontrolled diabetes increases AGE formation it follows that this will also lead to increased RAGE signalling, oxidative stress and inflammation, as outlined in Figure 4. Individuals with diabetic macrovascular complications have an 8-fold increase in RAGE mRNA expression compared to non-diabetic control patients and significantly higher serum AGE levels have been observed in diabetic patients with vascular complications[47]. This association of enhanced AGE concentrations with oxidative stress and diabetic complications suggests that measurements of serum AGE could be used clinically as a biomarker of vascular complications in diabetic patients.

A recent study has shown that 16 weeks' oral administration of mangiferin, an anti-inflammatory and anti-diabetic agent, was able to significantly ameliorate the burden of the AGE/RAGE interaction in diabetic rats by reducing RAGE expression and the consequent activation of NF-κB in the myocardium and production of inflammatory cytokines[48]. The authors suggested that mangiferin treatment could be beneficial in diabetic cardiomyopathy through inhibiting ROS accumulation and

the downstream damaging signalling events. Mangiferin supplementation for twelve weeks has been used successfully to improve serum lipid profiles in overweight individuals with hyperlipidaemia, without any adverse effects[49]. This approach may therefore be useful in treating diabetic cardiomyopathy through exerting systemic anti-inflammatory effects that could resolve over-activity of RAGE signalling in the heart and vasculature. However, there are currently no data available on the capacity of mangiferin to protect against cardiomyopathy in humans and it is clear that additional research is required in this area.

2.6.2. Interaction between RAGE signalling and other ROS generating pathways

As shown in Figure 4, AGE/RAGE cellular signalling leads to ROS generation and the activation of transcription factors, such as NF-κB. There is no consensus regarding the exact process by which ROS is generated through RAGE/AGE signalling, but evidence suggests that AGE binding to RAGE leads to activation of NADPH oxidase enzymes[45,50] and formation of the MPTP[51]. It is hypothesised that AGE/RAGE-derived ROS can interact with NADPH oxidase enzymes to potentiate further ROS synthesis, and that NADPH oxidase-derived ROS can have the same effect on AGE/RAGE-derived ROS in a positive feedback loop[45]. Exposure of RAGE-expressing human endothelial cells to AGEs led to enhanced oxidative stress and expression of tissue factor, indicating the presence of inflammation[50]. Furthermore, gp91phox knockout mouse macrophages did not exhibit any increase in tissue factor or oxidative stress on exposure to AGE, providing direct evidence that NADPH oxidase is involved in the generation of ROS through AGE/RAGE signalling. These observations suggest an alternative activation pathway of NADPH oxidase, secondary to RAGE activation, and therefore another potential pharmacological target for macrovascular and microvascular complications of diabetes.

In the hyperglycaemic state, increased glucose-derived NADH feeds into complex 1 of the ETC, and it has been observed that under these conditions of NADH excess AGE activation of RAGE elevates cytosolic ROS, which facilitates over-production of mitochondrial $O_2^{\cdot-}$[51]. These findings implicate ROS generation, caused by AGE/RAGE signalling in the hyperglycaemic state, as an upstream cause of mitochondrial ROS synthesis, indicating that pharmacological inhibition of this pathway may reduce oxidative stress and end-organ damage in uncontrolled diabetes. Given that AGE/RAGE signalling may act as a common upstream stimulus in ROS generating pathways, therapies targeting RAGE activation may be a sensible strategy for reducing oxidative stress to prevent or treat diabetic macrovascular complications. It should be noted that studies to date have mainly focused on the role of AGE/RAGE-induced oxidative stress in the pathogenesis of renal diabetic complications. Further investigation into AGE/RAGE signalling in the diabetic heart is required to ascertain whether this is a pathway of significance in this type of diabetic insult, and whether treatment targeted at preventing AGE action will be of benefit in diabetic CVD.

2.7. Protein Kinase C-mediated signalling

Protein kinase C (PKC) is a family of kinases that is involved in many cellular functions, including cell growth and death. Chronic activation of PKC under hyperglycaemic conditions leads to activation of a number of signalling cascades that can induce oxidative stress, including activation of NF-κB, increased activity of NADPH oxidases, overproduction of cytokines and induction of apoptosis, particularly in the vascular system. It is these alterations that have been linked to the pathogenesis of diabetic macrovascular disease.

2.7.1. Diacylglycerol generation de novo

Excessive production of $O_2^{\cdot}$ by the mitochondria under hyperglycemic conditions can inhibit the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which normally converts glyceraldehyde 3-phosphate into 1,3-diphosphoglycerate using nicotinamide adenine dinucleotide. When GAPDH is inhibited by $O_2^{\cdot}$, upstream glycolysis metabolites are forced into alternative pathways. In the case of PKC, glycerol 3-phosphate, derived from the glycolytic intermediate dihydroxyacetone phosphate, will be converted in a two-step process into DAG. Therefore, DAG levels are increased via this *de novo* synthesis pathway when glucose levels are elevated[52] and this can lead to increased DAG concentrations in the heart[53].

It has been found that expression of the DAG-sensitive PKC isoforms, PKCβ1 and PKCβ2, are up-regulated in human heart failure cardiomyocytes[54] and, consistent with this, PKCβ2 mRNA and protein expression in aortic endothelial and muscle cells were elevated in the hyperglycaemic state[53]. STZ-induced diabetes in transgenic mice over-expressing PKCβ2 in the myocardium (PKCβ2Tg) resulted in cardiac fibrosis that covered 2-3% of the left ventricle, while control mice had a total area of fibrosis of <0.1%[55]. Additionally, there was a 1.7-fold increase in collagen VI deposition in the ventricles of the diabetic PKCβ2Tg mice, which was completely alleviated by treatment with the PKCβ2 inhibitor[55]. These alterations in diabetic PKCβ2Tg mouse cardiac histology were thought to be a result of PKCβ2-mediated cytokine production in the myocardium[55], but as yet no causal relationship between PKC activation, cardiac hypertrophy and cytokine production has been established. Additional studies showed that PKC activation of in the mouse heart led to cardiac hypertrophy and dysfunction[56] and 2 to 10-fold increase in PKCβ2 mRNA in diabetic PKCβ2 transgenic mice correlated with LVH and reduced ventricular performance, while LY333531 significantly improved these parameters[57]. Overall, these studies support a role for PKC, in particular the PKCβ2 isoform, in mediating deleterious effects of hyperglycaemia on cardiac function.

3. The damaging effects of oxidative stress on the cardiovascular system

Increased phosphorylation of redox-sensitive kinases such as ERK1, ERK2 and JNK has been implicated in downstream signalling during oxidative stress. Significantly increased ERK1/2

phosphorylation has been identified in an animal model of LVH, and, as the LVH progressed, the level of ERK phosphorylation continued to increase[27]. These findings led to the proposal that the ROS generated in the myocardium acted via up-regulation of transcription factors that mediated the damaging effects. It is now well established that oxidative stress and PKC activation causes an increase in expression of NF-κB[19, 37, 58], a transcription factor that increases iNOS expression, leading to increased NO production. NO can react with O_{2^-} to generate peroxynitrite, which is capable of inducing the formation of the MPTP and resulting in further ROS generation, cytochrome c leakage and apoptosis[58]. The generation of ROS specifically from the myocardium has been implicated in further mitochondrial dysfunction and modification of mitochondrial DNA[21]. Damage to mitochondrial DNA leads to impaired synthesis of mitochondrial proteins and promotes further ROS generation[58].

Oxidative stress can cause damage to RNA as well as DNA. Recently, a human trial discovered that type 2 diabetic patients with macrovascular complications had elevated urine concentrations of 8-OH-dG, a marker of ROS-induced DNA damage (section 2.1.1), and levels of the RNA oxidation marker 8-OH-G were also significantly increased[59]. This study not only lends further support for oxidative damage to nucleic acids in diabetes, but introduces a potential new biomarker for untreated or undiagnosed diabetic complications which may have a clinical use in monitoring the progression of diabetic complications and their response to therapy.

NF-κB activation also increases the expression of several pro-inflammatory, pro-fibrotic and pro-hypertrophic genes. Cardiac inflammation is an important part of the pathogenesis of CVD, and it is a strong candidate for the mediator of ROS-induced cardiac fibrosis and hypertrophy in diabetic CVD. Indeed, patients with type 2 diabetes have a 1.5-fold increased risk of LVH than non-diabetic controls[60], and elevated concentrations of pro-inflammatory cytokines, such as IL-6 and TNF-α, have been recorded in patients with type 2 diabetes[61]. TNF-α caused enlargement of neonatal rat cardiomyocytes through increased generation of ROS, and this cardiac hypertrophy was abolished by administration of anti-oxidants[62]. Findings from further studies investigating the role of TNF-α in myocardial inflammation and fibrosis, specifically in diabetic cardiomyopathy, are consistent with this cytokine having a damaging effect on the heart. Thus, it has been found that treatment of diabetic rats with an anti-TNF-α antibody for six weeks reduced cardiac TNF-α expression by 2-fold, and this was accompanied by reductions both in cardiac IL-1β expression and ERK phosphorylation[63]. Inhibition of TNF-α in the diabetic rats was also associated with significant reductions in collagen deposition and improved LV function, indicating that TNF-α antagonism may have a role in diabetic cardiomyopathy therapy.

Oxidative stress in the diabetic myocardium is also associated with increased levels of 4-HNE (section 2.1.1). Administration of the anti-oxidant N-acetyl-cysteine abolished the elevation of 4-HNE and reduced expression of TNF-α and NF-κB, producing a concomitant reduction in collagen deposition, and therefore fibrosis[37]. This highlights that inflammation and fibrosis in diabetic

cardiomyopathy is caused, at least in part, by oxidative stress and illustrates the potential clinical use of anti-oxidants against diabetic cardiomyopathy.

4. Therapeutic potential of anti-oxidants in diabetic cardiomyopathy

The human body synthesises anti-oxidant enzymes to maintain the balance between ROS generation and degradation and while ROS play physiological functions under normal conditions it is generally accepted that excess ROS is harmful, as summarised above. A recent study has demonstrated that levels of the anti-oxidant glutathione peroxidase 3 are reduced with age, accompanied by an increased risk of adverse cardiovascular events[64] suggesting that preventing this decline or provision of exogenous anti-oxidants may be therapeutically beneficial. Several studies have investigated anti-oxidants as therapies for diabetic complications. Some of those that are relevant to protection against diabetic cardiomyopathy are summarised below, with their sites of action shown in Figure 7, and section 4.9 considers the therapeutic feasibility of these anti-oxidants.

4.1. Coenzyme-Q10

Coenzyme-Q10 is an endogenously synthesised anti-oxidant and a component of the mitochondrial ETC. Highly metabolic tissues such as the myocardium are abundant in mitochondria and therefore have high concentrations of coenzyme-Q10. A study in the hyperglycaemic db/db mouse model has led to the proposal that coenzyme-Q10 may be as effective as current therapies for diabetic cardiomyopathy given that its administration was associated with similar reductions in LV mass and collagen deposition to those obtained with the ACE inhibitor ramipril[65]. These observations in mice appear promising, but further investigations are required to determine whether exogenous provision of coenzyme-Q10 has beneficial effects to ameliorate cardiomyopathy in humans. Coenzyme-Q10 is currently available as a non-prescription oral dietary supplement, and a recent randomised controlled trial indicated that its use as an adjunct therapy for chronic heart failure was associated with a significant reduction in major adverse cardiovascular events[66]. There is currently no information on the utility of coenzyme Q-10 as a stand-alone therapy for heart failure, nor whether it is suitable for patients with diabetic cardiomyopathy.

4.2. Mito-TEMPO

The mitochondria-targeted anti-oxidant mito-TEMPO has been investigated as a potential treatment for diabetic cardiomyopathy in mice[67]. It was found that H_2O_2 generation was elevated in hyperglycaemic db/db mice and in mice that had been made diabetic by STZ administration, and daily injection of mito-TEMPO significantly reduced the hyperglycaemia-induced elevation in H_2O_2. In addition, mito-TEMPO administration in vitro protected against hyperglycaemia-induced cardiomyocyte death. Thus, this study is consistent with mito-TEMPO reducing cardiomyopathic

changes in the hyperglycaemic state in both type 1 and type 2 diabetic mouse models. Mito-TEMPO is targeted to the mitochondria, but it was also observed that it reduced mRNA expression of gp91phox and p47phox, components of NADPH oxidase (Figure 4). The findings from this study therefore provide further support of an interaction between NADPH oxidase and the mitochondria in the generation of ROS.

4.3 Peroxiredoxin-3

Peroxiredoxin-3 (Prx-3) is another mitochondrial anti-oxidant, which has recently been investigated in the context of hyperglycaemia-induced cardiac dysfunction in diabetic cardiomyopathy[68]. It was found that Prx-3 levels were significantly reduced in rat H9c2 cardiac cells maintained under hyperglycaemic conditions, and also in STZ-induced diabetic rats. The induction of diabetes in these rats led to increased cardiac 4-HNE, which was used as a marker of lipid peroxidation. Treatment of the diabetic rats with the naturally occurring flavonol quercetin resulted in elevation of endogenous Prx-3 such that normal Prx-3 levels in the myocardium were restored and cardiac troponin I levels were also increased, indicating alleviation of cardiac injury and improved cardiac function. Quercetin treatment also reduced 4-HNE levels towards normal and diabetes-induced remodelling of the heart, as determined by myocardial fibrosis, altered cardiac architecture and hypertrophic markers such as connective tissue growth factor, was attenuated with quercetin treatment. Overall, the data presented in this study indicate that increasing endogenous Prx-3 through administration of quercetin is cardioprotective, at least in rodents, but the therapeutic potential of this approach for treatment of cardiomyopathy in humans has not yet been investigated.

4.4 Rutin

Rutin, a glycoside of quercetin, exhibits anti-oxidant and anti-inflammatory properties. Its anti-inflammatory effects have been demonstrated in a recent study in which its intraperitoneal administration in a rat model of neuroinflammation significantly reduced expression of the inflammatory cytokines IL-1β, IL-6 and TNF-α, and it also diminished RAGE and NF-κB protein expression[69]. The effect of rutin to protect against diabetic cardiomyopathy has also been investigated, and it has been found that its administration to STZ-induced diabetic rats for 24 weeks resulted in reductions both in cardiac oxidative stress and cardiac inflammation[70]. The study also demonstrated that the total anti-oxidant capacity of the rutin-treated diabetic rats was significantly improved and their TNF-α expression was dramatically reduced. Additionally, histopathological observations of the hearts of the diabetic animals that had been treated with rutin established improved indices of cardiac hypertrophy. These findings in rats suggest a potential role for the anti-oxidant rutin in the pharmacological treatment of diabetic cardiomyopathy.

4.5 N-acetyl-L-cysteine

The glutathione precursor anti-oxidant N-acetylcysteine (NAC) has free radical scavenging properties and it has been reported that treatment of rat cardiomyocytes with NAC abolished TNF-α-induced ROS generation[71]. In vivo protective effects of NAC in diabetic cardiomyopathy have also been demonstrated. Thus, NAC administration after STZ-induced diabetes in mice led to significant attenuation of the cardiac fibrosis that had been caused by the diabetes, which was associated with a marked reduction in ROS formation[72]. The study highlighted that better cardiac outcomes were obtained when NAC was administered early after the induction of diabetes by STZ, indicating that timely therapeutic intervention is likely to be important in preventing long term cardiac damage that occurs with sustained hyperglycaemia.

NAC may also have beneficial effects through reducing NADPH oxidase activity, as has been reported for Mito-TEMPO (section 4.2), since diabetes-induced up-regulation of $p22^{phox}$, a crucial subunit of NADPH oxidase, was reduced in diabetic rats treated with NAC[73]. In the same study it was demonstrated that NAC-treated diabetic rat myocardium had significantly lower indices of oxidative stress. These results are consistent with NAC treatment conferring a reduced capability of the myocardium to generate ROS through the activity of NADPH oxidase and suggest that therapeutic treatment of diabetic cardiomyopathy with NAC may be of benefit by reducing cardiac oxidative stress and cardiac fibrosis, potentially by affecting the synthesis of NADPH oxidase subunits.

4.6 Sitagliptin

Sitagliptin is currently in clinical use as a diabetes therapy. It inhibits dipeptidyl peptidase-4 (DPP-4), the enzyme responsible for inactivating the incretin hormone glucagon-like peptide-1 (GLP-1), and sitagliptin is reported to be cardioprotective in a rat model with type 2 diabetes through maintaining endogenous GLP-1 levels[74]. More recently, it has become apparent that sitagliptin can protect against diabetic cardiomyopathy by down-regulating the JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway, which can cause cardiac hypertrophy through increased cell differentiation, growth, survival and angiogenesis[75]. It has recently been demonstrated that daily administration of sitagliptin via oral gavage to STZ-induced diabetic rats for 90 days led to a significantly reduced heart-to-body weight ratio, indicating reduced levels of cardiac hypertrophy[76]. In addition, sitagliptin administration to the diabetic rats caused marked improvement in activities of the anti-oxidant enzymes superoxide dismutase and catalase. Furthermore, cardiac homogenates of the sitagliptin-treated diabetic rats showed significantly reduced levels of lipid peroxidation and reduced myocardial cell degeneration and collagen deposition through attenuation of cardiac IL-6 levels. Immunochemical staining demonstrated that sitagliptin reduced phosphorylation of JAK2 and STAT3 in the diabetic rat heart. These findings indicate that sitagliptin can have cardioprotective effects in rat models of diabetes by impairing signalling through the JAK/STAT pathway. It was not investigated in

the study whether the reduced JAK/STAT phosorylation was secondary to elevations in GLP-1, but the observed reduction in IL-6 suggests inhibition of RAGE cascades, and it has been reported previously that sitagliptin impairs RAGE signalling[77]. There are no reports of use of DPP-4 inhibitors such as sitagliptin being associated with reductions in cardiac hypertrophy in humans, and further studies are required to determine whether they may have a role in the prevention or treatment of diabetic cardiomyopathy.

4.7 Nobiletin

Nobiletin is a citrus flavonoid that is reported to reduce the depletion of endogenous anti-oxidants such as glutathione, superoxide dismutase and catalase in rats in which acute renal injury was induced by administration of the oxidative stress-generating anti-cancer drug cisplatin[78]. Recent investigation into the therapeutic effects of nobiletin has indicated a potential role for this drug in diabetic cardiomyopathy. Thus, administration of nobiletin for 11 weeks to STZ-induced diabetic mice with cardiomyopathy led to improved LV function[79]. In addition, analysis of mRNA expression of NADPH oxidase subunits in cardiac tissue showed that nobiletin-treated diabetic mice had significant reductions in p22phox and gp91phox compared to the control diabetic mice, which may account for the lower levels of the lipid peroxidation marker malondialdehyde (MDA) with nobiletin administration. Additional exploration of the cellular mode of action of nobiletin showed that it significantly attenuated the diabetes-induced increases in TNF-α and IL-6 mRNA in the myocardium through reduced activation of NF-κB. These findings indicate that nobiletin may be able to abate oxidative damage in the diabetic myocardium by reducing hyperglycaemia-induced NADPH oxidase up-regulation, preventing excessive ROS accumulation and therefore impeding NF-κB activation and synthesis of pro-inflammatory cytokines.

4.8 Curcumin

Curcumin, isolated from turmeric powder, has known anti-oxidant, free radical scavenging and anti-inflammatory effects[80]. In a study in which curcumin was administered for 16 weeks to diabetic rats it was found that the indices of LV systolic function were markedly improved and cardiomyocyte hypertrophy was significantly alleviated[81]. The diabetes-induced increases in AGE accumulation and RAGE expression were attenuated with curcumin treatment and curcumin also protected against the increases in cardiac protein expression of p47phox and gp91phox. Measurements of MDA and superoxide dismutase activity indicated that curcumin therapy led to an overall reduction in oxidative stress. These findings are suggestive of multiple sites of beneficial action of curcumin: through action on AGE, RAGE and NADPH oxidase, supporting a therapeutic role for this anti-oxidant in diabetic cardiomyopathy. A recent meta-analysis of eight randomised controlled trials indicated that curcumin supplementation led to a significant reduction in circulating concentrations of the pro-inflammatory

cytokine TNFα, and the authors proposed that curcumin could be a cost-effective therapy for TNFα-associated diseases[82].

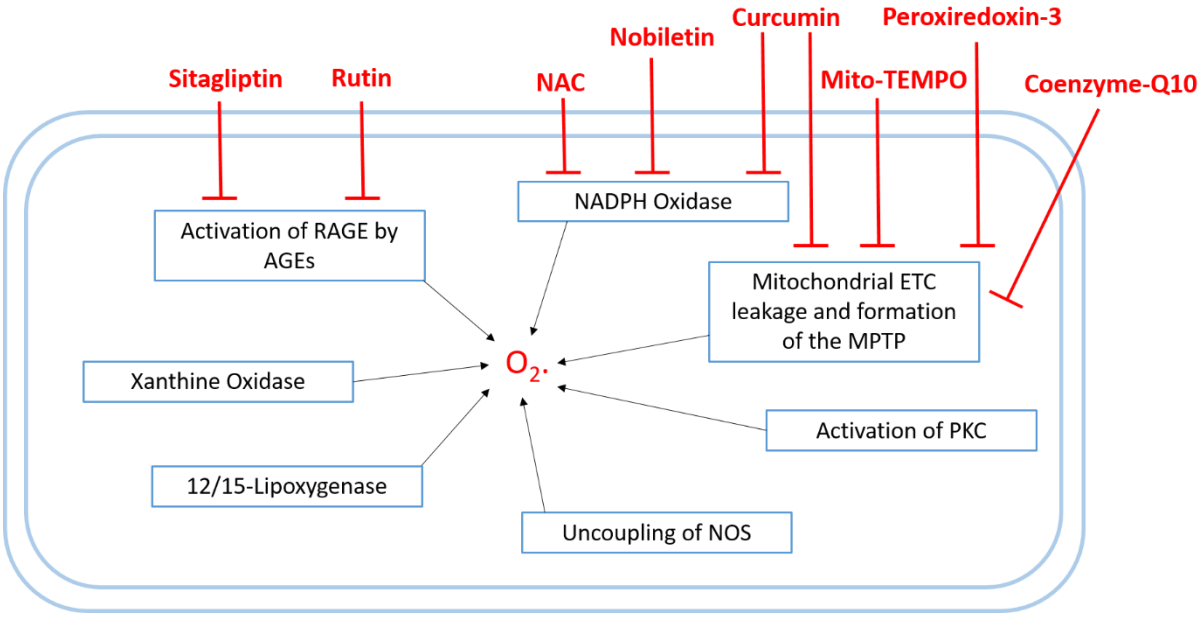

Figure 7. Sites of action of anti-oxidants
A variety of anti-oxidants have been tested in animal models of diabetic cardiomyopathy, and the figure summarises how they interact with the $O_2^{\cdot}$ generating pathways to protect against cardiac dysfunction.

4.9. Feasibility of therapeutic use of anti-oxidants for diabetic cardiomyopathy

The studies described above are generally supportive of the use of anti-oxidant therapies for diabetic cardiomyopathy, but they have been carried out in animal models in which a type 1 diabetic phenotype has been generated by STZ-induced beta-cell destruction or in the insulin resistant db/db mouse model of type 2 diabetes. It is not yet clear whether these rodent studies may be directly translated to the human situation, and consideration must be made of the potential for harmful long term effects such as increased risk of cancer[83] or higher mortality[84] that may occur with systemic administration of anti-oxidants. Targeting the heart through administration of therapeutically selective anti-oxidants is an attractive option, but there is no evidence of the existence of cardiac-specific molecules in the signalling cascades that promote the deleterious effects of oxidative stress and this is, at best, a remote future possibility. Nonetheless, well-tolerated systemic anti-oxidants such as curcumin, which have multiple beneficial effects and are well tolerated, may prove to be useful in reducing oxidative stress in diabetic cardiomyopathy.

The applicability of these anti-oxidants for treatment of human diabetic cardiomyopathy can be gauged, to a certain extent, by considering data obtained from meta-analyses of randomised controlled trials where some of these agents have been used for other conditions. Thus, there is

evidence that curcumin supplementation in humans can decrease circulating TNFα[82] and that quercetin can reduce blood pressure[85]. However, Cochrane reviews investigating other anti-oxidant therapies such as coenzyme Q10 for chronic heart failure[66] and hypertension[86], have given conflicting outcomes with suggestions of positive effects[66] or that its use is not associated with a clinically significant beneficial outcome[86]. This suggests that anti-oxidants are not generalised panaceas and that they may lead to improvements in some, but not all clinical conditions associated with oxidative stress. Furthermore, in some cases anti-oxidant use has been associated with harmful outcomes. For example, NAC administration for systemic inflammatory response syndrome (SIRS) and sepsis led to cardiovascular depression if it was given later than 24 hours after onset of symptoms[87]. However, it should be noted that in the trials evaluating the effectiveness of NAC for treating SIRS and sepsis it was delivered by intravenous infusion, while it was administered orally in mice where its effects on cardiac fibrosis were investigated[71,72].

It is also worth considering whether using anti-oxidants to combat increased oxidative stress in diabetes is the best strategy to treat diabetic cardiomyopathy. As stated in section 1.3, generation of ROS is an appropriate cellular response to maintain homeostatic balance and blocking this through the use of anti-oxidants could be deleterious. For example, in a study investigating the progression of atherosclerosis in diabetic mice it was demonstrated that although deletion of the NADPH oxidase isoform NOX4 led to a reduction in the ROS generating capacity of the mice there was actually an increase in atherosclerosis[88]. This therefore suggests that NOX4-derived ROS are protective against diabetic atherosclerosis, consistent with observations that NOX4 deletion led to increased apoptosis[88] and NOX4 overexpression improved endothelial function[89]. Thus, these studies challenge the traditional consensus of oxidative stress and an imbalance in cellular redox states leading to cell dysfunction through lipid, protein and DNA modification and support a new paradigm where ROS generated by the NOX4 isoform may protect against onset of disease. It is therefore clear that a more nuanced approach to anti-oxidant therapy for the treatment of diabetic cardiovascular disease is required, rather than a global “one size fits all” strategy.

5. Conclusions and future perspectives

Uncontrolled diabetes produces a chronic hyperglycaemic state that is associated with potentially fatal myocardial tissue damage. There have been thorough investigations on hyperglycaemia-induced generation of oxidative stress in diabetic CVD, and the consensus is that there is indeed an alteration in the oxidative state of the diabetic heart. Hyperglycaemia provokes the myocardium into excessive ROS production through a number of inter-related pathways, with research suggesting that the interaction between AGEs and RAGE is a common upstream pathway. Oxidative stress is highly damaging: it causes modification of lipids, proteins, DNA and it activates transcription factors that drive inflammation, fibrosis and hypertrophy. As such, therapeutic interventions to

decrease oxidative stress could, in theory, protect against hyperglycaemia-induced myocardial tissue damage. However, while the research presented here regarding the use of anti-oxidants, mainly in animal models of diabetes, appears promising, there is still insufficient information available on the significance of the sources of oxidative stress in different tissues or on the potential benefits and risks of systemically reducing oxidative stress in the diabetic state in humans. Further study into the integrated mechanisms that generate ROS in the diabetic heart and effectors that are responsible for the deleterious effects will provide greater insight into the pathogenesis of diabetic CVD and, potentially, lead to introduction of targeted therapies for this potentially fatal condition.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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