REVIEW

Diabetes Management

The role of reactive oxidative species in insulin resistance-associated cardiovascular disease

Noman Ali[1], Mark T Kearney[1] & Richard M Cubbon*[,1]

Practice points

- Insulin resistance is a crucial link between diabetes mellitus and cardiovascular disease.
- Oxidative stress, one of the recognized characteristics of insulin resistance, is instrumental in the development of endothelial dysfunction due to disruption of nitric oxide production.
- The processes of angiogenesis and vascular repair are of key importance with respect to mitigating the progression and sequelae of vascular damage.
- Murine studies have shown maintenance of reactive oxygen species levels within an optimal window to be necessary in order to facilitate effective endothelial regeneration.
- In the setting of insulin resistance, a reduction in reactive oxygen species levels has been shown to augment angiogenesis.
- Therapeutic translation of antioxidant strategies have so far proved disappointing but the concept of NADPH oxidase-specific inhibitors shows promise.

SUMMARY Due to an alarming growth in prevalence, diabetes mellitus (DM) has attained the status of a global epidemic. Most deaths linked to DM are attributable to cardiovascular disease, and the elevation in cardiovascular risk in DM is thought to be underpinned by resistance to the vascular and metabolic effects of insulin. One of the characteristics of insulin-resistant states is the excessive production of reactive oxygen species. Recent studies have highlighted this to be a crucial contributor to the relationship between insulin resistance and cardiovascular disease, leading to research investigating the potential therapeutic implications of manipulating reactive oxygen species levels in the context of insulin resistance. This review summarizes our current understanding and future direction in this emerging field.

Due to an alarming growth in prevalence, diabetes mellitus (DM) has attained the status of a global epidemic, and WHO predicts that DM will be the seventh leading cause of death by 2030 [1]. Most deaths linked to DM are attributable to cardiovascular disease (CVD) and while great advances have been made in CVD prevention among the general population, outcomes remain poor for patients with DM, who tend to suffer with complications such as myocardial infarction (MI) or stroke approximately 15 years prematurely [2]. While hyperglycemia is the diagnostic criterion, and an important target in the treatment of DM, cardiovascular risk is also significantly elevated even in the context of prediabetes when sustained hyperglycemia is not apparent [3]. This is thought to be due to an aggregation of cardiovascular risk factors, referred to as the metabolic syndrome, which may be underpinned by resistance to the vascular and metabolic effects of insulin.

KEYWORDS
• angiogenesis
• atherosclerosis
• cardiovascular disease
• diabetes mellitus
• endothelial dysfunction
• insulin resistance
• oxidative stress • reactive oxygen species • vascular repair

[1]Leeds Institute of Cardiovascular & Metabolic Medicine, LIGHT laboratories, The University of Leeds, Clarendon Way, Leeds, LS2 9JT, UK
*Author for correspondence: Tel.: +44 113 343 0785; Fax: +44 113 343 7738; r.cubbon@leeds.ac.uk

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Insulin resistance is implicated in the initiation of atherosclerosis, the key pathological process responsible for CVD. By disrupting endothelial nitric oxide (NO) production [4], insulin resistance is instrumental in the development of endothelial dysfunction, recognized as the earliest manifestation of atherosclerosis [5]. Furthermore, insulin resistance may facilitate the progression of atherosclerosis by impairing endogenous vascular repair processes, which can retard atherogenesis and promote recovery of damaged endothelium in the context of vascular injury [6,7].

One of the characteristics of insulin-resistant states is excessive production of reactive oxygen species (ROS) [8], which has been demonstrated to be a key pathogenic process in endothelial dysfunction [9] (see **Figure 1)**. Recent work has also highlighted the critical role of ROS as signaling molecules in the process vascular repair [10]. ROS could therefore form a target for both prevention and treatment of insulin resistance-associated CVD. This brief review aims to outline current understanding in the field, focusing particularly on the advanced CVD associated with insulin-resistant syndromes, but also briefly discussing the role of ROS early in the disease continuum.

Insulin signaling & resistance

Insulin signaling is achieved via its interaction with the insulin receptor. Binding of insulin to its receptor induces autophosphorylation of intracellular tyrosine residues, leading to recruitment and phosphorylation of insulin receptor substrate and other docking proteins. This initiates multiple downstream signaling cascades, the principal two being the PI3K/Akt and MAPK pathways. Activation of the PI3K/Akt pathway promotes GLUT-4 translocation in metabolic tissues and NO production via endothelial nitric oxide synthase (eNOS) activation in vascular tissues [4].

The term 'pathway-specific' insulin resistance describes the phenomenon observed in the vasculature of obese prediabetic rodents, whereby activation of the PI3K pathway by insulin is impaired, while MAPK activity remains unaffected [11]. The net result of this imbalance is to promote the mitogenic effects of insulin while hindering its ability to stimulate NO production. As mentioned above, the reduction in vascular NO bioavailability is an instrumental link between insulin resistance and CVD.

Vascular repair & angiogenesis

The culminating event in atherosclerotic disease, such as MI or stroke, is the rupture of a plaque formed by arterial wall inflammation and lipid accumulation, resulting in acute vascular occlusion. Endogenous vascular repair mechanisms are responsible for re-endothelialization of the blood vessels injured by the formation and rupture of plaques. Meanwhile, downstream tissues rendered ischemic by vascular occlusion depend on the formation of neovessels for their reperfusion. Angiogenesis is the term given to the sprouting or intussusception of neovessels from pre-existing vasculature and is the key process involved in postischemic neovascularization [12].

While angiogenesis is the predominant means of generating capillary beds in adulthood [13], *de novo* blood vessel formation, termed vasculogenesis, is also of great significance during embryonic development. Until recently this process was thought to be exclusive to the prenatal stage, but work conducted by Asahara *et al.* [14] challenged this notion with the discovery of 'putative progenitor endothelial cells'. These culture-expanded circulating CD34-expressing cells were found to display an endothelial-like phenotype *in vitro*, while augmenting angiogenesis when reinfused into mice with hind-limb ischemia. Further studies in humans have suggested that the abundance of circulating cells expressing CD34 in conjunction with other putative progenitor cell surface markers independently predict future major cardiovascular events [15]. Many other studies using various populations of cell culture expanded 'endothelial progenitor cells' (EPCs) have suggested a potential role in vascular repair and angiogenesis [16] and in more recent years, there has been a significant body of research focusing on the relationship between EPCs, ROS and vascular regeneration. Leroyer *et al.* [17] postulated that in the setting of ischemia, ROS produced by endothelial cell-derived microparticles mediate the differentiation of the progenitor cells which facilitate postnatal vasculogenesis. However, perturbations in ROS levels have been shown to have detrimental effects with regard to EPC function. Sambuceti *et al.* [18] identified the excess of ROS characteristic of Type 2 DM as being responsible for the impaired capability of the vascular wall to recruit EPCs. ROS also play a similarly complex role in developmental vasculogenesis – while beyond the scope of this

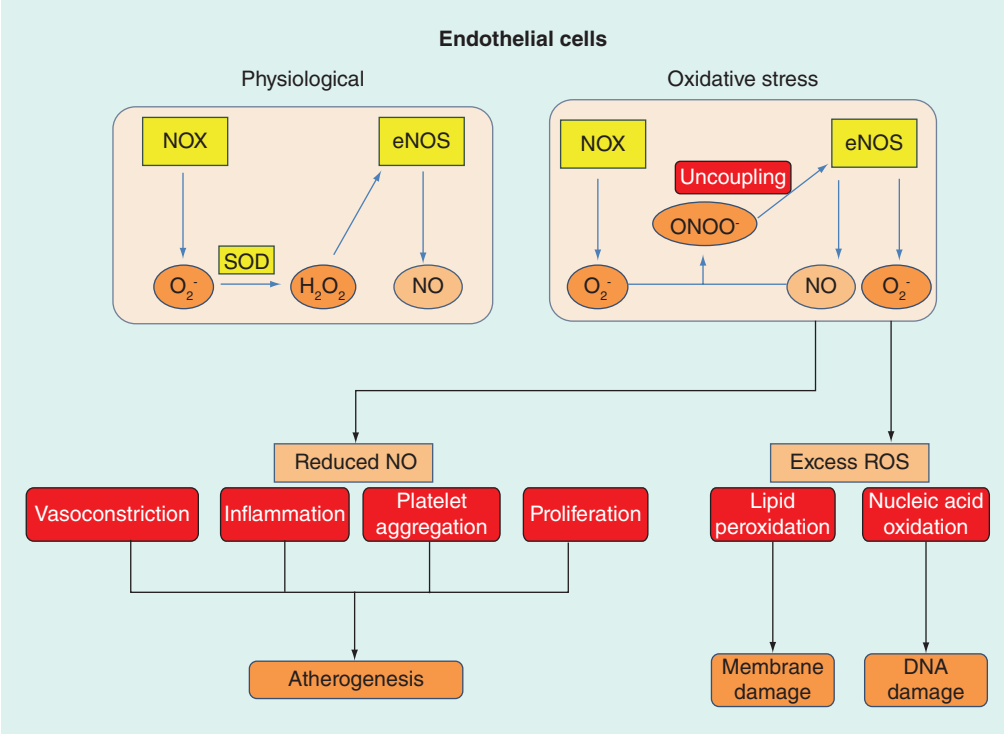

Figure 1. Reactive oxygen species and endothelial dysfunction. In health NADPH oxidase produces superoxide anion which is dismuted into hydrogen peroxide by the action of SOD. H_2O_2 can promote activation of eNOS, increasing NO bioavailability, which maintains endothelial function and is antiatherogenic. In the setting of oxidative stress, excess production of O_2^- results in sequestration of NO via a reaction to produce peroxynitrite. $ONOO^-$ oxidizes the eNOS cofactor BH_4, uncoupling eNOS to then produce further O_2^-. This prevents the action of soluble guanylyl cyclase and leads to impaired vasorelaxation. The reduction in NO bioavailability also leads to inflammation, platelet aggregation and cell proliferation, all of which are contributory factors to atherogenesis. The excess ROS can also react with cellular macromolecules such as lipids and nucleic acids resulting in damage to membranes and DNA.
H_2O_2: Hydrogen peroxide; eNOS: Endothelial nitric oxide synthase; O_2^-: Superoxide anion; $ONOO^-$: Peroxynitrite; NO: Nitric oxide; ROS: Reactive oxygen species; SOD: Superoxide dismutase.

review, other comprehensive reviews exist on this subject [19].

While there remains significant debate regarding the existence and role of EPCs *in vivo*, there is a general acceptance that such cells represent a potential autologous cell therapy to promote vascular repair and neovascularization [20].

Reactive oxygen species

The term 'ROS' encompasses various molecules derived from the metabolism of oxygen which can be divided into free radicals (superoxide anion, peroxynitrite) and nonradicals (hydrogen peroxide, ozone). ROS levels within cells are governed by the relative rates of their production and breakdown. The most significant producers of ROS are mitochondria and various enzyme groups including xanthine oxidase, lipoxygenase, uncoupled eNOS and the NADPH oxidases (NOX). The lattermost of these is the principal source of ROS within the vasculature and will be discussed in more detail later. Breakdown of ROS is also attributable to the actions of a selection of enzymes. Superoxide anion is dismuted into hydrogen peroxide and oxygen via the action of superoxide dismutase (SOD), while hydrogen peroxide breakdown into water and oxygen is facilitated by catalase, glutathione peroxidise (Gpx) and the thioredoxin enzyme system (see **Figure 2**) [21].

In health, production and breakdown are balanced and a physiological level of ROS is therefore maintained. In situations where there is excessive production or insufficient breakdown

of ROS, accumulation occurs and this is termed 'oxidative stress'. Excess ROS cause damage to cell constituents such as lipids, proteins and DNA due to free radical-mediated chain reactions [22]. While an excess of ROS is damaging, at physiological concentrations they play a crucial role with regard to normal cell growth, differentiation, migration and apoptosis [23]. A significant body of recent work has also highlighted the importance of ROS as intracellular signaling molecules. Signal transduction mediated by ROS, known as 'redox signaling', has been demonstrated to be crucial to numerous developmental and physiological processes [22].

• NADPH oxidase

In contrast to all other ROS producing enzymes, the NOXs produce ROS as their primary function, rather than as a byproduct [24]. NOX2 is the prototypic NADPH oxidase, and was first discovered in phagocytes, where it aids in antimicrobial defense by producing a 'respiratory burst' of ROS to kill internalized bacteria [19]. Subsequent work found NOXs to also be present in nonphagocytic cells, leading to the discovery of a family of NOXs. We now recognize there to be five NOX homologs (NOX 1-5) and two dual oxidases (Duox 1-2) [25].

NOX2 is the most widely expressed NADPH oxidase isoform within the vasculature [26] and has also been identified in stem/progenitor cells [25]. Structurally, it consists of a membrane spanning catalytic subunit termed gp91phox, which in order to function requires binding of the membrane bound p22phox subunit, as well as further cytosolic components (p47phox, p67phox, p40phox and Rac) [9].

NOXs 1 and 4 are also expressed within the vasculature. NOX1 shares a number of similarities with NOX2, including structural homology (60% shared aminoacid identity), the fact that its primary product is superoxide anion and its activity's dependence on binding with other subunits [27]. NOX4 contrasts with NOXs 1 and

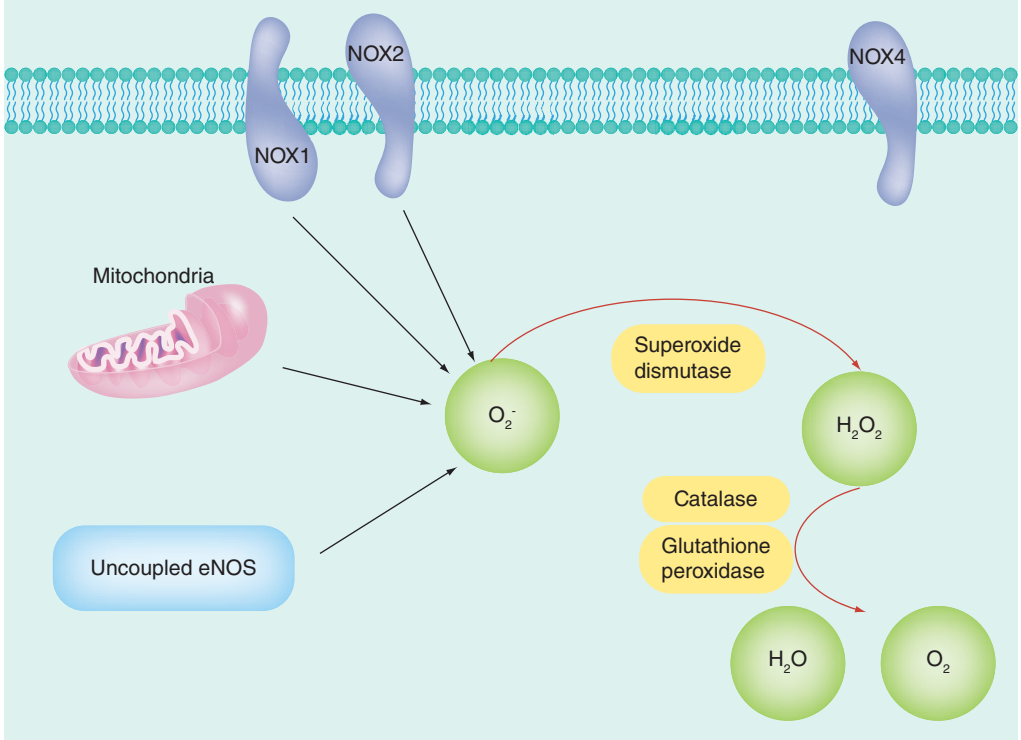

Figure 2. Sources and breakdown of reactive oxygen species. Membrane-bound NADPH oxidase enzymes (NOXs 1 and 2) as well as mitochondria and uncoupled eNOS are responsible for the majority of superoxide anion generation. Superoxide anion is dismuted to form hydrogen peroxide by the action of the enzyme superoxide dismutase. NADPH oxidase NOX4 also contributes to hydrogen peroxide generation, which is then itself broken down into water and oxygen by the actions of catalase or glutathione peroxidase.
eNOS: Endothelial nitric oxide synthase.

2 in that it appears to generate predominantly hydrogen peroxide rather than superoxide anion and that it is constitutively active, thereby not requiring cytosolic activator subunits [9]. This suggests that its ROS production is dependent on enzyme abundance, and so is regulated predominantly by level of its expression [28].

The role of ROS in insulin resistance-associated vascular disease

As alluded to in the introduction of this review, DM is associated with greater cardiovascular risk than can be explained by hyperglycemia alone, and a number of papers have provided evidence implicating insulin resistance as one aberration responsible for this observation. A study undertaken by Yip *et al.* demonstrated that in nonobese nondiabetic patients, insulin resistance is a predictor of the development of CVD, independent of other risk factors [29]. In order to dissect the role of ROS in the link between insulin resistance and vascular disease, our lab has studied a number of murine models which aim to represent the prediabetic phenotype. In 2007 we published a study [30] demonstrating that mice haploinsufficient for the insulin receptor (IRKO) displayed accelerated endothelial dysfunction, relative to wild-type (WT) controls, in spite of preserved glucose regulation. The study also found that use of a SOD mimetic could restore endothelial function in IRKO mice, which were shown to generate greater quantities of ROS than WT counterparts. While this study examined genetically induced insulin resistance, there is also evidence to show that ROS have a role to play in the insulin resistance and endothelial dysfunction that is observed secondary to diet-induced obesity [31]. The cited paper demonstrated that middle-aged mice fed with a high fat diet (HFD) developed obesity, insulin resistance, dyslipidemia, hypertension and endothelial dysfunction associated with increased NOX2 expression and ROS production. Inhibition of NOX2, either genetically or pharmacologically, was shown to reverse all of the HFD-induced abnormalities except for hyperinsulinemia. The conclusion from the paper was that NOX2-derived ROS played a key role in damaging insulin receptor and endothelial function in dietary obesity after middle age.

Given the close relationship between insulin resistance and endothelial dysfunction, as well as the pivotal role of the endothelium in process of vascular regeneration, we have undertaken further work focusing on the effect of endothelial-specific insulin resistance. Transgenic mice with endothelium-targeted overexpression of a dominant-negative mutant human insulin receptor (ESMIRO) were generated and their endothelial function examined. ESMIRO mice displayed normal glucose regulation, but impaired endothelial function, associated with reduced NO bioavailability, which could be rescued *in vitro* with a SOD mimetic [32]. Furthermore, when ESMIRO mice were cross-bred with atherosclerosis-prone ApoE-defficient mice, the resulting ApoE$^{-/-}$/ESMIRO mice exhibited accelerated atherosclerosis [7]. This finding has been independently corroborated by Rask-Madsen *et al.* who demonstrated that ApoE$^{-/-}$ mice with endothelial insulin receptor knockout had increased atherosclerosis relative to ApoE$^{-/-}$ mice [33].

In the aforementioned endothelium-specific insulin resistance studies performed by our group, the combination of elevated levels of ROS, and the ability of pharmacological superoxide diminution to reverse the observed endothelial dysfunction, implicates oxidative stress as an underlying mechanism. This suggests that NOX2, the primary generator of superoxide anion within the vasculature, could form a target for reducing oxidative stress and the associated endothelial dysfunction in insulin resistance. To this end our group has generated transgenic ESMIRO mice with global NOX2 knockout (ESMIRO/NOX2$^{y/-}$) and demonstrated that genetic NOX2 inhibition could reduce superoxide levels and improve vascular function [34]. These findings were replicated with use of either acute or chronic pharmacological NOX2 inhibition. This study established NOX2 inhibition as a novel therapeutic target in insulin resistance-associated vascular dysfunction and further research is underway to explore the specific effect of this on vascular repair and angiogenesis. Importantly, NOX1 (but not NOX4) has also been implicated as another translational target in diabetes-associated atherosclerosis [35], and endothelial dysfunction, potentially via eNOS uncoupling [36]. However, it is also likely that the sources and abundance of ROS evolve during the transition from prediabetes to frank DM [37].

The role of ROS in angiogenesis & vascular repair

Research in the field of redox signaling has highlighted the role of ROS as signaling molecules

within pathways crucial to the processes of angiogenesis and vascular repair. The resulting implication that inhibition of their action in a physiological setting would be detrimental to these processes has been corroborated in multiple *in vivo* studies. Schroder *et al.* [10] found that, while re-endothelialization of injured mouse carotid artery is enhanced by hypoxia, this effect is abrogated in NOX2$^{y/-}$ mice. Further data from this study went on to link this finding to a deficiency in hypoxia-induced EPC mobilization in the NOX2$^{y/-}$ mice, which could be restored by the transplantation of WT bone marrow (BM). Similarly, deleterious effects have also been demonstrated from studies looking into the effect of NOX2 knockout on angiogenesis. Tojo *et al.* [38] found NOX2$^{y/-}$ mice to have impaired angiogenesis relative to WT controls and these findings were replicated by Urao *et al.* [39], who went on to show that the impaired angiogenesis seen in NOX2$^{y/-}$ mice could be ameliorated by the transplantation of WT BM. Importantly, NOX4 knockout mice have also been shown to exhibit endothelial dysfunction and impaired angiogenesis [28,40].

While these studies demonstrate that inhibiting NADPH oxidases in 'healthy' mice setting impairs angiogenesis, when considering human disease states and potential therapeutic strategies, manipulation of ROS in models better reflecting clinical disease is also important. In 2006, Ebrahimian *et al.* [41] published a paper indicating that mice with Type 1 diabetes have impaired postischemic neovascularization relative to WT littermates. They went on to show that reducing ROS in these mice by knocking out NOX2 could restore the angiogenic capacity back to WT levels. The finding that NOX2 deletion has a protective effect on ischemic limb angiogenesis in the context of oxidative stress has also been corroborated by Haddad *et al.* in two recent studies of cigarette smoke exposure [42] and diet-induced hypercholesterolemia [43]. In both studies, NOX2 deletion was shown to be beneficial to angiogenesis with the proposed mechanism being preservation of the proangiogenic vascular endothelial growth factor NO signaling pathway. The same group has more recently noted that NOX2 deletion can rescue ageing associated reduction in recovery from hind-limb ischemia, potentially via enhanced EPC-mediated angiogenesis [44].

Less has been published with regards to the role of ROS in insulin resistance-associated diminished vascular repair. Our group has demonstrated that IRKO mice, shown previously to be a model of oxidative stress [30], display impaired vascular endothelial regeneration following arterial injury when compared with WT littermates [6]. Further investigation into the cause of this aberration found the IRKO mice to be deficient in circulating progenitor cells due to defective mobilization from the BM, secondary to reduced eNOS expression. In addition to reducing the number of progenitor cells, there is evidence in the literature to suggest that oxidative stress can also impair their function. Sorrentino *et al.* [45] published a study demonstrating that EPCs derived from individuals with diabetes have a severely impaired capacity for re-endothelialization when infused into mice following carotid artery injury. Reduction of ROS production in these EPCs by siRNA silencing of the p47phox NOX2 subunit reversed the impairment, suggesting that the maintenance of ROS levels within an optimal range is crucial for ensuring adequate reparative function of progenitor cells. The potential role of other NOX isoforms in insulin resistance-associated vascular disease and impaired vascular regeneration remains unexplored.

The finding that a reduction of ROS levels can be either detrimental or beneficial depending on absence or presence of oxidative stress is best explained using the 'redox window' hypothesis which was first put forward by Yun *et al.* [46] when studying redox signaling in coronary collateral growth. It states that a hypothetical window exists within which 'redox state not only permits, but also can amplify coronary collateral growth. A shift in the redox state to either to an overly-reductive environment or an oxidative environment corrupts growth factor-initiated redox-dependent signaling'. Reducing ROS levels below a certain threshold (into an 'overly-reductive environment') via deletion of NADPH oxidase appears to be detrimental to vascular regeneration. However in situations of underlying oxidative stress, such as diabetes, a reduction in ROS has been shown to augment angiogenesis by shifting the redox state from an oxidative environment back into the hypothetical optimal window (see **Figure 3**). Support for this concept in human disease comes from the recent work of Ali *et al.* [47], who showed that genetic variants associated with reduced expression of Gpx1 are associated with risk of arterial neointima formation. Surprisingly, they noted

that beyond the somewhat predictable oxidative stress and enhanced atherosclerosis seen in Gpx1 knockout mice, glutathione accumulation resulted in simultaneous 'reductive stress', manifesting in the form of signaling dysregulation caused by inhibitory S-glutathionylation of the phosphatase SHP-2. This illustrates the complexity of redox imbalance in human disease, and supports the need for nuanced understanding of disease mechanisms in order to produce effective therapies.

ROS & other CVD risk factors

Beyond promoting endothelial dysfunction and impaired vascular regeneration, oxidative stress is also associated with risk factors for developing CVD (for a more detailed review see Roberts *et al.* [48]). A number of studies have demonstrated oxidative stress to be a contributory factor in the development of hypertension; in the context of angiotensin II-infused mice, NOX2 inhibition has been shown to normalize blood pressure [49] and conversely, endothelial-specific NOX2 overexpression to promote hypertension [50]. However, NOX2 may not mediate renin and endothelin-1-induced hypertension [51,52]. Conversely, endothelial NOX4 overexpression has also been shown to reduce blood pressure due to the vasodilatory action of hydrogen peroxide [53]. Small-scale clinical trial data also illustrate the potential of broad-spectrum antioxidants to improve insulin sensitivity, adipokine profile, and indices of leukocyte–endothelial interaction over the short term in overweight young adults [54]. However, as discussed later, these promising findings have not translated into reductions in 'hard outcomes' in large clinical trials.

While papers cited in this review article have highlighted a role for ROS in atherogenesis in the context of insulin resistance, there is also recognition that oxidative stress can independently promote the development and progression of atherosclerosis. An interesting, recently published paper quantified subclinical atherosclerosis in patients with chronic granulomatous disease (CGD), an immunodeficient state most often caused by defective gp91phox (NOX2). Despite a high prevalence of traditional cardiovascular risk factors, patients with CGD exhibited reduced carotid artery atherosclerosis relative to age-matched controls [55]. The findings of this study support a role for NOX2-derived ROS in the pathogenesis of atherosclerosis and although the mechanism was not explored in this paper, insights can be gained from previously published work. For example, oxidative stress is known to induce the expression of proinflammatory adhesion and chemotactic molecules such as vascular adhesion molecule 1 (VCAM-1) [35] and MCP-1 [56], which in turn promote the recruitment and accumulation of inflammatory cells to atherosclerotic lesions [57,58].

Therapeutic implications

Given the multitude of preclinical studies that highlight the potential of manipulating ROS to prevent or treat disease processes associated with insulin resistance, it is of no surprise that interest in the therapeutic application of antioxidants has been intense. However, clinical trials have thus far been disappointing. The initial strategy involved use of SOD mimetics with the intention of restoring oxidative balance. While this appeared to work well in laboratory studies, clinical efficacy was found to be poor [59]. Initial promise was also seen with the antioxidant vitamin E, which was found to reduce the incidence of MI in two small trials [60,61]. However, further research provided evidence to the contrary with a number of larger trials showing vitamin E to be either neutral [62–64], or even harmful [65].

The presumed reasons for the unsatisfactory results seen so far with antioxidants are multiple [66]; firstly, when antioxidants such as vitamin E scavenge radicals, they themselves become radicals with the associated potentially deleterious effects. Evidence also exists that oxidation of orally administered tetrahydrobiopterin prevents it from reducing vascular oxidative stress or limiting eNOS uncoupling [67]. Secondly, ROS production is specifically localized within cells and it is likely that the antioxidants are unable to effectively and specifically reach the required sites. Finally, and most significantly, the antioxidant approaches described thus far were nonselective in their scavenging of ROS. Given the importance of physiological levels of ROS in redox signaling, the damage caused to homeostatic processes by a blanket reduction in their levels may outweigh any potential benefit to the disease processes being targeted. Moreover, as discussed earlier, it is possible that oxidative and reductive stress occur simultaneously within cells, suggesting that instead of broad-spectrum redox modulation, more nuanced targeting of common pathophysiological processes may be required [47].

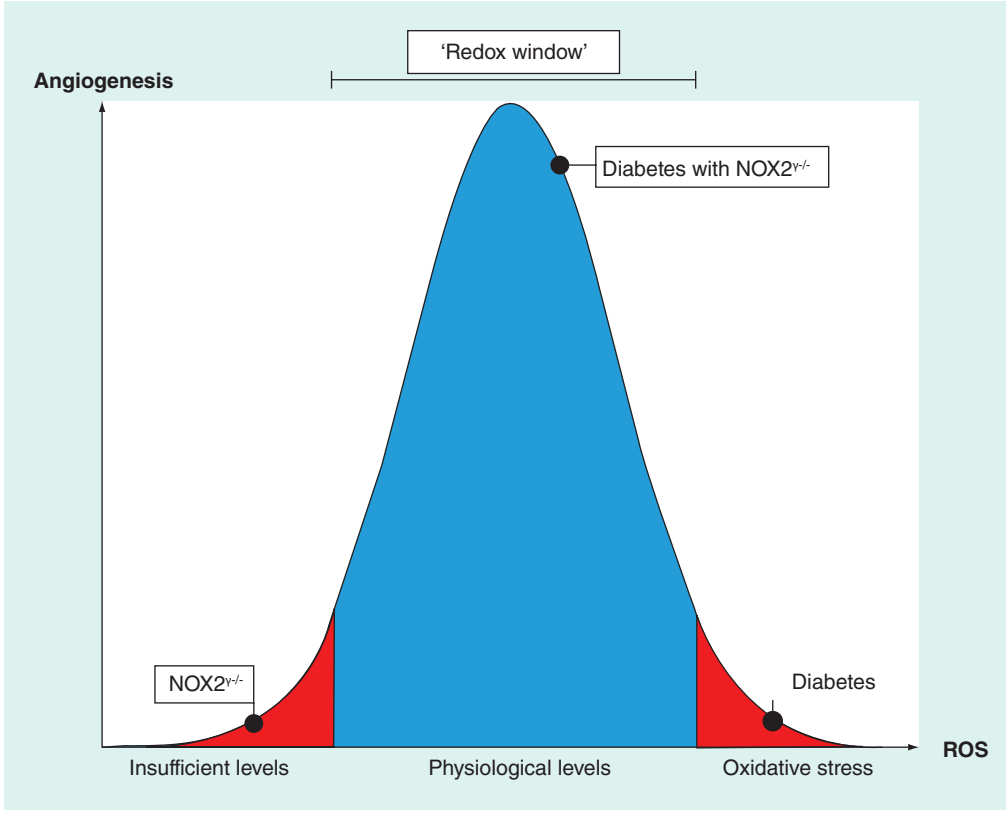

Figure 3. The 'redox window' hypothesis. NOX2 knockout results in impaired angiogenesis since insufficient levels of ROS are disruptive to redox signalling. However, when NOX2 is knocked down in the context of diabetes, angiogenesis can be augmented due to a shift from oxidative stress into the optimal redox window.
ROS: Reactive oxygen species.

Based on the issues identified with nonselective ROS reduction, research has turned to the therapeutic application of NADPH oxidase-specific inhibitors [68]. This area shows a great deal of potential, but a number of challenges remain. The most widely studied NADPH oxidase inhibitors are apocynin and diphenyleneiodonium, and while they have been of great value in preclinical research, their lack of specificity for NADPH oxidases over other enzymes limits their potential in clinical practice. The synthetic peptide GP91ds-tat shows more promise, in that it is a more specific NOX2 inhibitor, but the fact that it is a peptide presents issues with regard to its parenteral administration and pharmacokinetic profile. Another potentially significant issue with NOX2 inhibition is its role within the innate immune system, where it is responsible for destroying internalized pathogens with 'respiratory bursts' of ROS. There exists an anxiety that curtailing this immune function will compromise a patient's immune function in a manner analogous to the inherited immunodeficiency CGD.

Taking all of the above into account, one attractive solution appears to be a specific small molecule NOX2 inhibitor which is able to maintain ROS levels within the 'redox window' whereby vascular function and repair is improved, but homeostatic and immune functions are not compromised. Further research is necessary in order to better characterize NOX2 biology and the extent of enzymatic blockade required in order to achieve the desired reduction of ROS.

Conclusion & future perspective

Since the initial discovery that ROS could be damaging to cells and their constituents, our understanding has substantially evolved, painting a much more nuanced and complex picture. While oxidative stress has been proven to be a fundamental contributor to the initiation and progression of multiple disease processes, including diabetes-associated CVD, we also know that

ROS have a crucial role as mediators of cell signaling. Thus the concept of ROS as a double-edged sword emerges, whereby deviation from tightly regulated temporospatial 'redox window' is detrimental.

Pharmacological manipulation of ROS remains an attractive therapeutic strategy since it offers the opportunity to augment vascular function and regeneration, potentially revolutionizing the prevention and management of insulin resistance-associated CVD. However, this important goal has proven elusive in previous clinical trials, and many significant challenges remain if the exciting findings of recent preclinical studies are to achieve clinical impact.

Although broad-spectrum antioxidants have proven ineffective in preventing major cardiovascular events, a large body of preclinical and clinical data support the role of oxidative stress in the pathogenesis of insulin resistance-associated CVD. Emerging data suggest that specific sources of ROS may be implicated in the enhanced atherogenesis associated with insulin resistance, and that these sources may evolve in the transition to manifest DM. Moreover, it is now clear that ROS are also essential for normal vascular homeostasis. Therefore, we speculate that future clinical trials will need to apply therapies targeted at specific sources of ROS, which aim to achieve partial rather than complete inhibition of ROS production.

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• of interest; •• of considerable interest

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