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REVIEW

Clinical implications of oxidative stress and potential role of natural antioxidants in diabetic vascular complications

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Abstract *Aims:* The possible link between hyperglycaemia-induced oxidative stress (OxS) and diabetic complications is suggested by many in vitro studies. However, not much attention has been paid to the clinical evidence supporting this hypothesis, as well as to their possible therapeutic implications.
Data synthesis: Some prospective studies show a direct correlation between an increase in OxS biomarkers and the appearance of diabetes complications. This is consistent with the evidence that any acute increase of glycaemia, particularly post-prandial, and hypoglycaemia causes endothelial dysfunction and inflammation, through the generation of an OxS. However, the detection of free radicals is difficult as they are highly reactive molecules with a short half-life. Instead, the metabolites of OxS are measured. Interventional trials with supplemented antioxidants have failed to show any beneficial effects. Conversely, natural foods show very promising results.
Conclusions: The "new antioxidant" approach includes the possibility of controlling free radical production and increasing intracellular antioxidant defence, a concept different from the old one, when antioxidant activities implied scavenging the free radicals already produced. A synergistic action in this respect could convincingly be obtained with a balanced 'Mediterranean Diet' (MedD) type. Early intensive glucose control is still the best strategy to avoid OxS and its associated diabetes complications.

Abbreviations: AGEs, advanced glycation end products; RAGEs, AGE receptors; AT-II, angiotensin-II; CVD, cardiovascular disease; DN, diabetic nephropathy; DR, diabetic retinopathy; eNOS, endothelial nitric oxide synthase; FPG, fasting plasma glycaemia; iNOS, induced nitric oxide synthase; MedD, Mediterranean diet; MI, myocardial infarction; NADPH, nicotinamide adenine dinucleotide phosphate reduced; NEAC, non-enzymatic antioxidant capacity; NOS, nitric oxide synthase; NF-κB, nuclear factor kappa B; OxS, oxidative stress; PUFAs, polyunsaturated fatty acids; PPG, post-prandial glycaemia; PKC, protein kinase C; ROS, reactive oxygen species; RONS, reactive oxygen and nitrogen species; RNS, reactive nitrogen species; $O_2^{\bullet-}$, superoxide anion; TNF-α, tumour necrosis factor α; T1D, type 1 diabetes; T2D, type 2 diabetes.

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Oxidative stress

Oxidants generally comprise reactive oxygen and nitrogen species (RONS) [1]. While reactive oxygen species (ROS) result from an incomplete reduction of oxygen, reactive nitrogen species (RNS) are products of nitric oxide synthases (NOSs). Both are highly chemically reactive molecules, produced as a result of intracellular and extracellular stimuli; the latter acts as receptor agonists. The most significant sources of RONS are mitochondrial electron transfer chain and enzymatic systems as nicotinamide adenine dinucleotide phosphate reduced (NADPH), NOS, xanthine oxidase [2–6] and cytochrome P-450 system [7].

At the mitochondrial level, the reactive species are produced as 'by-products', and the NADPH oxidases primarily produce reactive species [8].

NADPH oxidases represent a family of enzymes and have seven isoforms, including NOX1-5 and DUOX1-2, based on a different core catalytic subunit. These isoforms present different distribution (NOX4 is the most widely distributed) and mechanisms of activation and their function is not completely known. NADPH oxidases produce superoxide (mainly by NOX1, NOX2, and NOX5) and/or hydrogen peroxide (mainly by NOX4) as intermediates of redox reactions [9].

There is a continuous interaction between these sources of reactive species: for example, uncoupling of eNOS (endothelial NOS) through oxidation of its cofactor leads to the production of peroxynitrite from nitric oxide generated by NOS and from superoxide generated by NADPH oxidases.

Moreover, superoxide produced by NADPH oxidases damages the inner mitochondrial membrane leading to alterations of electron transport chain, which in turn, enhances reactive species production [10].

RONS were considered as 'detrimental' or toxic by-products until their crucial roles in physiology have been described. It is currently known that RONS either initiate or act as the intermediate in several gene- and enzyme-dependant reactions in different normal or pathological conditions. RONS act as signalling molecules between mitochondria and other cellular compartments, which are required to promote health and longevity [11].

The imbalance between RONS production and the antioxidant defence, in favour of pro-oxidants, causes oxidative (OxS) or/and nitrosative stress. [12] A continuous high-level production of reactive species has a toxic effect and causes irreversible cellular damage by altering proteins, lipids, carbohydrates and DNA through the oxidation of amino acids and polyunsaturated fatty acids (PUFAs) [13].

The alterations in redox balance lead to a common pathway for many diseases including hypertension, atherosclerosis, heart failure and ischaemia reperfusion injury [14].

It is difficult to measure the levels of RONS, as they are highly reactive molecules with a short half-life. Consequently, both in research and clinical practice, the metabolite levels of OxS are measured, rather than reactive species.

OxS and vascular diabetic complications

The link between hyperglycaemia, OxS and diabetic complications has been shown previously [15].

Hyperglycaemia is characterized by a high-level production of superoxide. This not only causes direct damage to the cells but also activates four major pathways associated with diabetes complications: polyol pathway, increased production of advanced glycation end products (AGEs), activation of protein kinase C (PKC) isoforms and hexosamine pathway [16,17]. Furthermore, free radicals have the ability to activate several other pathways, which in turn induce an endothelial dysfunction [17].

It has been demonstrated that even a short-term exposure to hyperglycaemia selectively increases iNOS (induced NOS) gene expression, followed by a rise in NO. The endothelial cells are permeable to glucose through GLUT1 receptor in hyperglycaemic conditions; this increased intracellular supply of glucose will generate energy and high quantities of $O_2^{\cdot-}$ (superoxide anion) at the mitochondrial level. This simultaneous increase in NO and $O_2^{\cdot-}$ generates peroxynitrite. A significantly higher level of production of $O_2^{\cdot-}$ compared to NO leads to the inactivation of NO and endothelial dysfunction. Besides, peroxynitrite is a potent oxidant with a toxic effect on vasculature, which may contribute to the disease progression and myocardial damage [18].

However, besides the increased production of reactive species following hyperglycaemia, the *intracellular antioxidant defence* is of great importance in disease progression and development of diabetes complications [19].

Mitochondria and NADPH oxidases are the most important sites for reactive species production responsible for the CV complications in diabetes.

NOX1, NOX2, NOX4 and NOX5 are expressed in the endothelial cells; NOX1, NOX4 and NOX5 are expressed in the vascular smooth muscle cells; and NOX2 and NOX4 are expressed in the adventitial fibroblasts [10,20]. Other isoenzymes are expressed at very low levels or were not found at this level [21].

Reactive species have beneficial effects on the CV system as regulators of vascular tone and cell differentiation, migration and proliferation [10]. Conversely, NOX1 and NOX2 are responsible for the development of endothelial dysfunction, hypertension and inflammation, and NOX5 causes atherosclerosis [21]. NOX4 protects the vasculature against reactive species but is harmful in OxS conditions [21].

AGEs/RAGEs (AGE receptors), PKC and angiotensin-II (AT-II) activate NADPH oxidases and increase reactive species formation, and in OxS conditions, they lead to CV complications, including cardiac dysfunction. This process is facilitated by inflammation, fibrosis and apoptosis [22].

Several studies confirm, in vivo, in both normal healthy controls and diabetic patients that hyperglycaemia, particularly acute hyperglycaemia, causes OxS and subsequently endothelial dysfunction and inflammation. The role of OxS in the development of vascular diabetic complications is also confirmed by prospective studies in both type 1 (T1D) and type 2 diabetes (T2D) [23–27].