Challenging the dogma of mitochondrial reactive oxygen species overproduction in diabetic kidney disease

Melinda T. Coughlan[1,2,3] and Kumar Sharma[4,5,6]

[1]Baker International Diabetes Institute (IDI) Heart and Diabetes Institute, Melbourne, Victoria, Australia; [2]Department of Medicine, Central Clinical School, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria, Australia; [3]Department of Epidemiology and Preventive Medicine, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria, Australia; [4]Center for Renal Translational Medicine, Division of Nephrology-Hypertension, Institute of Metabolomic Medicine, University of California-San Diego, La Jolla, California, USA; [5]Division of Medical Genetics, Department of Medicine, University of California–San Diego, La Jolla, California, USA; and [6]Division of Nephrology-Hypertension, Veterans Affairs San Diego Healthcare System, La Jolla, California, USA

The paradigm that high glucose drives overproduction of superoxide from mitochondria as a unifying theory to explain end organ damage in diabetes complications has been tightly held for more than a decade. With the recent development of techniques and probes to measure the production of distinct reactive oxygen species (ROS) *in vivo*, this widely held dogma is now being challenged with the emerging view that specific ROS moieties are essential for the function of specific intracellular signaling pathways and represent normal mitochondrial function. This review will provide a balanced overview of the dual nature of ROS, detailing current evidence for ROS overproduction in diabetic kidney disease, with a focus on cell types and sources of ROS. The technical aspects of measurement of mitochondrial ROS, both in isolated mitochondria and emerging *in vivo* methods will be discussed. The counterargument, that mitochondrial ROS production is reduced in diabetic complications, is consistent with a growing recognition that stimulation of mitochondrial biogenesis and oxidative phosphorylation activity reduces inflammation and fibrosis. It is clear that there is an urgent need to fully characterize ROS production paying particular attention to spatiotemporal aspects and to factor in the relevance of ROS in the regulation of cellular signaling in the pathogenesis of diabetic kidney disease. With improved tools and real-time imaging capacity, a greater understanding of the complex role of ROS will be able to guide novel therapeutic regimens.

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Correspondence: *Kumar Sharma, University of California–San Diego, La Jolla, California 92093-0711, USA. E-mail: kumarsharma@ucsd.edu*

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The kidney is composed of multiple cell populations and is involved in a variety of essential regulatory functions such as blood pressure regulation, maintenance of acid-base balance, regulation of electrolytes, reabsorption of nutrients, and hormone secretion.[1] It is in part due to complexity of this organ that the pathogenesis of diabetic kidney disease (DKD) is difficult to decode. Traditional key pathways of pathology include an abnormally active renin angiotensin system, overproduction of reactive oxygen species (ROS), advanced glycation endproducts, enhanced endoplasmic reticulum (ER) stress, and proinflammatory cytokine signaling; however, targeted therapies have not led to clinical benefit, except for renin angiotensin system blockers.[2,3] However, recent studies[4–6] using unbiased approaches have provided new insights into DKD and highlighted the role of mitochondrial function in the pathogenesis of DKD. The exact role of mitochondrial function may be very different than the traditional role of mitochondria to provide excess ROS.[4–6] An unbiased interpretation of mitochondrial bioenergetics in association with superoxide production from intact mitochondria *in vivo*[7] will help to deconvolute the complex interplay of glucose, fatty acid, and amino acid oxidation and provide links to inflammation and fibrosis.

The historical view of ROS overproduction in DKD

The paradigm that increased oxidative stress may be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes and was first put forward by Baynes (1991).[8] Baynes suggested that diabetic microvascular complications are associated with increased chemical modification of proteins and lipids and that this "damage" appears to be largely oxidative in origin and sufficient to explain the altered function of proteins in the extracellular matrix.[8] The possible sources of oxidative stress and damage to proteins in diabetes were speculated to include free radicals generated by autoxidation reactions of sugars and sugar adducts to protein and by autoxidation of unsaturated lipids in plasma and membrane proteins. The specific constituents of ROS included superoxide, hydrogen peroxide, and lipid peroxides, precursors to more reactive species such as

hydroxyl radicals. Brownlee later refined this hypothesis by putting forward the view that the source of oxidative stress was derived from mitochondrial superoxide.[9] According to the Brownlee theory, excess glucose uptake by susceptible cells (cells which could not down-regulate excess glucose entry) would lead to an increase in pyruvate uptake into the mitochondria and an increased flux of substrates (nicotinamide adenine dinucleotide hydrate [NADH] and flavin adenine dinucleotide [FADH2]) to the electron transport chain (ETC),[9,10] resulting in hyperpolarization of the mitochondrial membrane and accumulation of electrons at complex III and coenzyme Q, which donate electrons to molecular oxygen and generate superoxide anions.[10] Whereas an increase in ROS-induced damage in the diabetic kidney is well supported by a large body of evidence (reviewed in Forbes *et al.*[4]), increased mitochondrial superoxide production does not appear to be consistently observed in target tissues of diabetes (recently reviewed in Sharma[7]). This may be, in part, due to the difficulty of measuring mitochondrial superoxide or may be due to an actual reduction of ETC activity and thus a lower production of superoxide from the ETC. This view does not preclude other sources of ROS as being up-regulated and playing harmful roles. In addition, it has also been recognized that there is a dual nature of ROS molecules, which are now known to mediate cross talk between a multitude of cell signaling pathways in a beneficial manner.[11,12] At present, we lack detailed understanding of the complex and dynamic interrelationships between these factors, particularly in the context of DKD.

Sources of ROS

Within the mammalian cell there are several sources of ROS (Figure 1), including those generated within the mitochondrion, ER, and peroxisomes and from within the cytosol including the enzyme systems xanthine oxidase, lipoxygenase, nitric oxide synthase, and nicotinamide adenine dinucleotide phosphate oxidase (NOX).[12] In particular, mitochondria are thought to generate a large proportion of intracellular ROS with the respiratory complexes, including flavoproteins, iron-sulfur clusters, and ubisemiquinone, contributing about 80% of superoxide in the basal state.[13] However, mitochondria contain both ETC-linked and non-ETC-linked pathways of ROS production.[14] Using isolated mitochondria, it has been shown that complex I and complex III of the ETC are major sites of superoxide production, occurring both on the matrix side of the mitochondrion and in the inner mitochondrial membrane space, whereas enzymes in the mitochondrial matrix, such as 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase, and the mitochondrial membrane forms of glycerol 3-phosphate dehydrogenase and the electron transfer flavoprotein-ubiquinone oxidoreductase mitochondrial system also produce superoxide.[14,15]

Envisioning that mitochondria will be functioning at a high level of efficiency with limited caloric supply in the basal state will support the hypothesis that superoxide production will be continuously generated under normal and fasting glucose conditions. Several factors regulate superoxide production in mitochondria, including the concentration of enzyme- or protein-containing electron carriers able to react

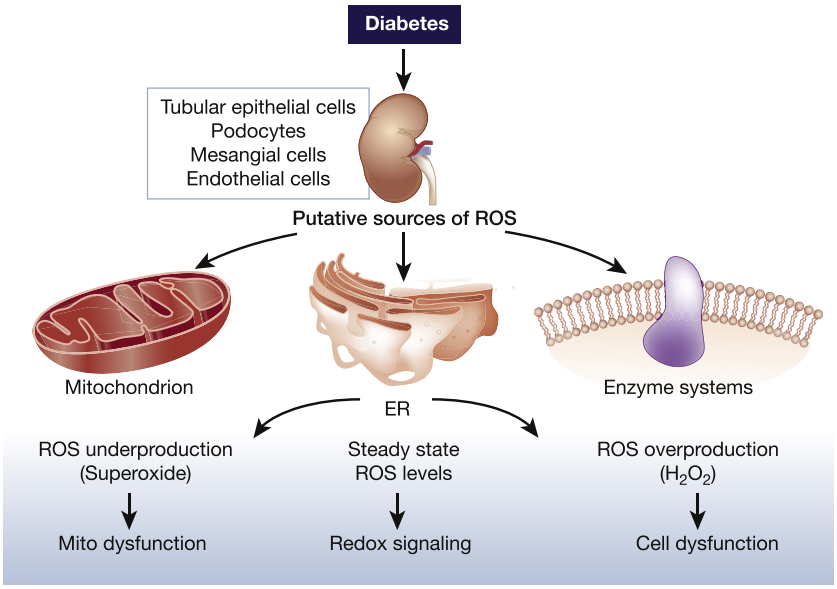

Figure 1 | The differential role of ROS in the pathogenesis of diabetic kidney disease. Putative sources of ROS in diabetic kidney disease include ROS production from various organelles within the cell including mitochondria, the ER (particularly in the setting of ER stress), and enzyme systems such as Nox. Key cells of the kidney that are likely to produce ROS include tubular epithelial cells, podocytes, mesangial cells, and endothelial cells. ROS underproduction, in particular superoxide, may indicate mitochondrial dysfunction or reduced function. Steady-state levels of ROS are likely physiologic and involved in normal cellular signaling, whereas excess ROS production can lead to cell dysfunction. *In vivo*, in diabetic kidney disease, the spatiotemporal production of ROS is not yet known. ER, endoplasmic reticulum; Mito, mitochondrial; Nox, reduced nicotinamide adenine dinucleotide phosphate oxidase oxidase; ROS, reactive oxygen species. Illustrations adapted from Servier Medical Art by Servier, used under Creative Commons Attribution 3.0 Unported License.