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Discoveries

FORUM REVIEW ARTICLE

Obesity and Diabetic Kidney Disease: Role of Oxidant Stress and Redox Balance

Kumar Sharma[1,2]

Abstract

Significance: Reactive oxygen species (ROS) reactive nitrogen species (RNS) and redox processes are of key importance in obesity- and diabetes-related kidney disease; however, there remains significant controversy in the field. ***Recent Advances:*** New data from imaging and *in vivo* models of obesity and diabetic kidney disease have shed new insights into this field. In the setting of obesity- and diabetes-related kidney injury, there is a growing recognition that the major moieties of ROS and RNS are hydrogen peroxide and peroxynitrite with the enzymatic sources being NADPH oxidases and nitric oxide synthase, respectively. However, the role of mitochondrial superoxide as a driver of renal complications remains unclear. ***Critical Issues:*** Several key issues that are often not discussed are the specific ROS and RNS molecules, the source of generation, the location of production, and downstream targets. ***Future Directions:*** Further understanding of the role of ROS/RNS/redox and their relationship with key signaling and metabolic pathways such as AMP-activated protein kinase (AMPK) and hypoxia-inducible factor 1-α (HIF1α) will be critical to a new understanding of kidney complications of caloric challenges and new therapeutic approaches. *Antioxid. Redox Signal.* 25, 208–216.

Introduction

THE USE OF AEROBIC OXYGEN took on paramount importance as eukaryotic and multicellular organisms developed a benefit from using oxygen for more efficient adenosine triphosphate (ATP) generation and providing evolutionary advantages over other life forms. The necessity for ATP generation took on greater significance as specific cell types became responsible for specialized tasks that were required to support multicellular organisms. As fish developed the ability to extract oxygen from the water for efficient utilization of carbon sources for ATP generation, they were able to form more complex structures with specific organs. With life forms adapting to life on land, oxygen extraction was switched from gills and water to lungs and air. Indeed, the rise in oxygen concentration was beneficial for organisms with efficient means of using oxygen for survival and evolutionary advantage, whereas anaerobic organisms would not be benefited. The Great Oxidation Event is a term used to describe the first mass extinction of anaerobic organisms and the rise in eukaryotes and multicellular organisms on earth (7, 38) about 2.2 billion years ago in concordance with a rise in atmospheric oxygen (Fig. 1).

Oxygen was not viewed as a toxic molecule until the recognition that oxygen radicals were developed and used as a host defense and killing mechanism by bacteria and eukaryotic cells to defend and attack potential invaders. Over the past six decades, the concept that reactive oxygen species (ROS) are toxic molecules and should be suppressed and scavenged has taken on greater popularity in the lay media and in the scientific community. The Free Radical Theory of Aging suggests that oxidants can be harmful and was initially met with scientific skepticism (21). Indeed there has been a recent major revision in the view that free radical oxidant molecules are a major cause of aging (46, 47, 49). However, the scientific community has largely accepted the theory that mitochondrial superoxide is a major unifying theory for diabetes- and obesity-related complications (41, 42). Specifically in obesity-related and diabetes-associated kidney disease, the concept that oxidant production is a major

[1]Center for Renal Translational Medicine, Institute of Metabolomic Medicine, University of California San Diego, La Jolla, California.
[2]Division of Nephrology-Hypertension, Veterans Affairs San Diego Healthcare System, La Jolla, California.

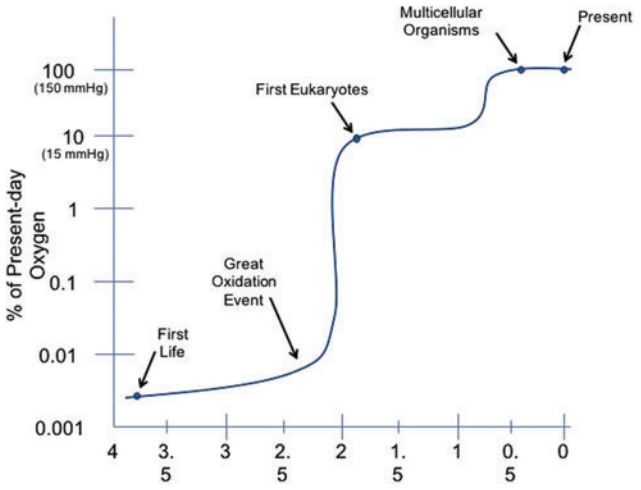

FIG. 1. Oxygen in the atmosphere and evolutionary complexity of organisms. Ancient photosynthetic prokaryotes (cyanobacteria) released oxygen first into the ocean, then into the atmosphere. The building of oxygen allowed the evolution of bigger eukaryotes and then multicellular organisms. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

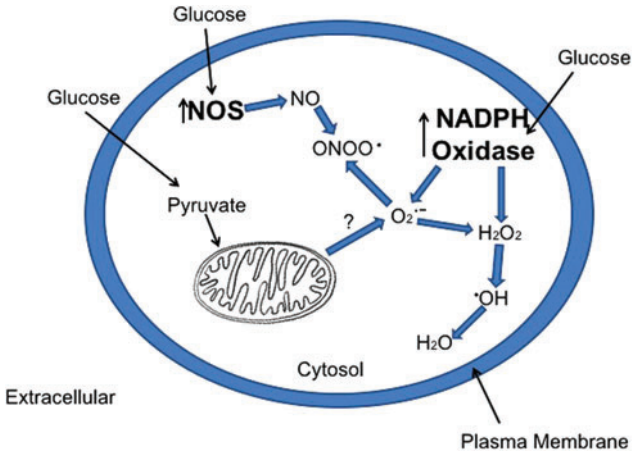

FIG. 2. Major sources of superoxide, hydrogen peroxide, and peroxynitrite in response to elevated glucose. The major sources of ROS production in response to excess intracellular glucose are possibly NOSs, NOXs, and mitochondria. The production of peroxynitrite from NOS and hydrogen peroxide from NOX are consistently observed, whereas measurement of superoxide is difficult to measure; therefore, the mitochondrial source is unclear. H_2O_2, hydrogen peroxide; NO, nitric oxide; NOS, nitric oxide synthase; NOXs, NADPH oxidases; $O_2^{\bullet-}$, superoxide anion; $ONOO^{\bullet}$, peroxynitrite; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

unifying theory for the development and progression of kidney disease has been widely accepted. The acceptance of this theory is somewhat surprising as several negative studies have been reported that do not support the theory, and in some cases even suggest that reducing ROS production in a nonspecific manner may be actually harmful (9, 19, 68).

In the present review, the role of oxidants and redox molecules as a cause of diabetes- and obesity-related kidney injury is examined and suggestions for more direct investigations are put forth to delineate the possible role of specific ROS molecules in the development and progression of nutrient-induced kidney disease. The term ''nutrient-induced kidney disease'' includes the features of both obesity-related and diabetes-associated chronic kidney disease.

Redox Balance, ROS, and Kidney Disease

The most commonly accepted definition of redox reactions includes all chemical reactions in which atoms have their oxidation state changed; in general, redox reactions involve the transfer of electrons between chemical species. The chemical species from which the electron is stripped from is ''oxidized'', whereas the chemical species to which the electron is added is ''reduced''. For the purpose of this review, the series of reactions linked to oxidant production is examined with their connection to kidney diseases linked to diabetes and obesity.

ROS and reactive nitrogen species (RNS) can be considered as unstable oxygen- or nitrogen-containing molecules that have a reactive electron or are an intermediate that can generate reactive molecules. The O_2 or H_2O molecules are considered to be relatively stable forms of oxygen. Normally, superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite are ''oxidizing agents'' that can oxidize a variety of key molecules and alter their function. Accordingly, oxidation of proteins, nucleic acids, lipoproteins, and phospholipids may be harmful in a specific context. The major sources of ROS include mitochondria (which are thought to provide about 80% of endogenously produced superoxide at the basal level), NADPH oxidases (NOXs), and nitric oxide synthases (NOSs) (Fig. 2). The precise amount and type of oxidant molecules produced by these sources under various metabolic conditions remain controversial as the methods to specifically measure each reactive oxidant moiety are not very quantitative and few have been applied in real-time *in vivo* states. Thus, much of the field relies on indirect measures of the footprint of oxidant production.

In almost all published studies that examine measures of ROS in diabetic tissue, there is a uniform overproduction of ROS molecules in the tissues of diabetic animals and patients (23, 26, 45). We too have found similar results, thus there is little doubt that overall ROS production is increased in diabetic tissues. Similar data are found in models of obesity as well (10, 44). However, several major questions have emerged in recent years regarding which species of reactive oxygen are increased, and what is the source of increased ROS in diabetic tissues and with obesity. These questions are largely unresolved. To address these questions we begin with an understanding of the major source of superoxide generation in relation to mitochondrial oxidative phosphorylation.

Oxygenation, Glucose, and Fatty Acid Oxidation in Kidney and Heart

Glucose metabolism begins with glycolysis and leads to formation of pyruvate from glucose in enzymatic steps not requiring oxygen. Glycolysis is a relatively ancient pathway and is exclusively used in anaerobic organisms (Fig. 3). The glycolytic steps leading to lactate and ATP production are

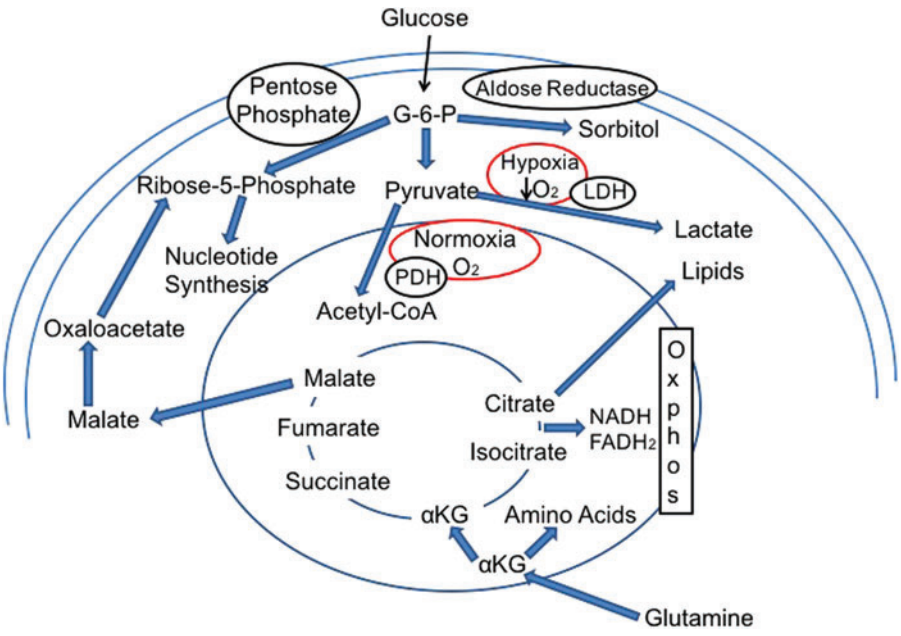

FIG. 3. Glucose metabolism in presence of oxygen and with low oxygen. Glucose metabolism is primarily *via* glycolysis leading to pyruvate formation and further metabolism in the tricarboxylic acid (TCA) cycle within the mitochondria. Alternative paths include metabolism *via* the pentose phosphate shunt pathway and aldose reductase pathway. With hypoxic conditions there is enhanced pyruvate to lactate glycolysis and reduced pyruvate uptake into the mitochondria. With an elevation in intracellular glucose and a hypoxic or pseudohypoxic reduction in OxPhos activity, there could be a backup of TCA cycle metabolites leading to enhanced citrate conversion to lipid synthesis, αKG leading to amino acid synthesis, and malate leading to nucleotide synthesis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

relatively fast and may be more prominent in states of high-glucose entry even under normoxic conditions (59). Facultative anaerobic organisms are organisms that can make ATP in the presence of oxygen *via* aerobic respiration and revert to fermentation or anaerobic respiration in the absence of oxygen (Fig. 3). Mammalian cells are generally considered facultative anaerobes and can switch to anaerobic glycolysis or aerobic glycolysis depending on the availability of oxygen and particular challenges. The transference of electrons is a critical step involved in aerobic oxidation of glucose within the mitochondria and specifically within the electron transport chain (ETC).

Fatty acid oxidation also requires oxygen and takes place in the mitochondrial and peroxisomal compartments. The role of fatty acid oxidation has been well studied in the heart as the heart generally prefers beta-oxidation of fatty acid for ATP generation under baseline euglycemic and well-perfused states. The heart uses the majority of oxygen consumption for fatty acid oxidation as opposed to glucose oxidation (33, 39). Although there is a greater generation of ATP per gram from fatty acids as opposed to glucose, the relative efficiency of oxygen utilization for ATP generation actually favors glucose oxidation. Cardiac metabolism is primarily aerobic and ATP is generated *via* oxidative phosphorylation of fatty acids and glucose. During transient and mild ischemia as well as diminishing oxygen supply, there is a proportionate reduction in oxidative phosphorylation, although fatty acid oxidation remains the primary source of ATP generation. There is an increase in the ATP production from glycolysis in an effort to maintain the energy needs for continued contractility, but cellular integrity may be compromised partly because of increased lactate and hydrogen ion generation from glycolysis (34). The balance between energy needs and control of cytoplasmic and mitochondrial metabolites has been termed ''metabolic homeostasis.'' In heart tissue, the regulation of metabolic homeostasis involves a complex interplay of cytoplasmic and mitochondrial networks that regulate the rate of ATP production over a wide range of workloads. Although there is remarkable flexibility under well-perfused conditions, there is a tremendous challenge under conditions of ischemia. The additional challenge of diabetes and obesity presents an additional set of variables that are yet to be resolved.

In the kidney, the relative roles of glucose and fatty acid oxidative phosphorylation and glycolysis are even more complex as there are multiple cell types with different propensities for glucose and fatty acid metabolism under the baseline euglycemic, well-perfused state. Using *in vitro* studies or based on transcriptomic profiles of the major enzymes involved (hexokinase, phosphofructokinase, and pyruvate kinase), glycolysis appears to be largely present in the inner medulla and papilla. Stimulation of the pentose phosphate shunt is initiated by glucose 6-phosphate dehydrogenase (G6PD). As this enzyme is present throughout the nephron and at high levels in glomeruli and proximal tubule, it is possible that there is high activity of this pathway in these segments. In one study, using C1–C6 labeled glucose consumption, the pentose phosphate shunt accounted for 10% of glucose utilization in tissue slices and various nephron segments (48). Cortical glycolysis leading to production of lactate appears negligible in the rat kidney under fasting conditions, whereas there is evidence of ongoing glycolytic lactate production in the medullary segments (48). There also appears to be a relationship between glycolysis and sodium reabsorption in the thick ascending loop of Henle.

Glucose oxidation is also preferentially greater in medullary segments than proximal segments. Based on flux studies with $^{3}H_2O$ and $^{13}CO_2$ generation, the ratio of glucose oxidation was found to be 3:1 in medullary tubules *versus* cortical tubules (48). The absolute amount has been estimated to be about 25 μmol/h/g dry weight in cortical tubules and between 100 and 180 μmol/h/g in medullary tubules. Although proximal tubules have a low ability to undergo glycolysis and glucose oxidation at basal states, the relative contributions of

glycolysis and glucose oxidation are unclear under conditions of high glucose or diabetes. In contrast to glucose oxidation, the proximal tubule preferentially undergoes fatty acid oxidation much more so than distal or medullary segments (48). The proximal tubule is considered to be a major source of mitochondrial beta-oxidation of free nonesterified fatty acids and is the major source of ATP production. Oxidation of fatty acids has been identified to account for two-thirds of the overall oxygen consumption of the kidney. A recent study demonstrated that reduced fatty acid oxidation is a feature of advanced kidney disease and is linked to profibrotic pathways (28); however, the metabolic pathways that characterize the initial and late stages of disease with different nephron segments have not been well described. The regulation of these metabolic pathways in disease states will be important to identify whether oxidant stress is generated *via* mitochondrial or nonmitochondrial sources.

ROS Production in Diabetes- and Obesity-Related Kidney Disease

Excess mitochondrial superoxide production as a unifying cause of diabetic complications was espoused by Brownlee (42) and has been widely accepted. The theory is that cells susceptible to noninsulin-dependent glucose uptake will metabolize excess glucose *via* glycolysis and that pyruvate uptake into mitochondria will be enhanced. The increased activity of the tricarboxylic acid cycle will lead to increased production of electron donors, and *via* enhanced ETC activity there will be an excess of superoxide production. Although it is quite clear that there is likely enhanced glucose flux and increased glycolytic and possibly enhanced tricarboxylic acid (TCA) activity in cells and tissues exposed to high glucose (8, 61), the question of increased ETC activity and superoxide production emanating from the ETC remains in doubt. The initial studies by Brownlee and colleagues used aortic endothelial cells in culture to demonstrate increased ROS production by 2′,7′-dichlorofluorescein (which primarily measures H_2O_2 and not superoxide), and by using inhibitors of the ETC, they found that downstream pathways of protein kinase C (PKC), advanced glycation end-products, and sorbitol were reduced (42). However, similar studies were not performed *in vivo* (Fig. 4).

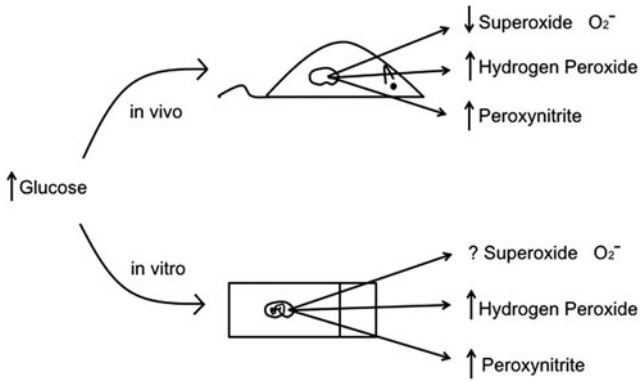

FIG. 4. Comparison of findings on major ROS molecules in the *in vivo* state and *in vitro* cell culture in response to elevated glucose. In the *in vivo* state and *in vitro* state, there is an increase in H_2O_2 and peroxynitrite. However, in the *in vivo* state, there is a reduction in superoxide with type 1 diabetic models, whereas the regulation of superoxide by high glucose *in vitro* is unclear.

A major concern with *in vitro* studies is the change in oxygen tension from the *in vivo*, which will alter mitochondrial oxidative metabolism in a dramatic manner. These studies raise doubt whether data generated from cell culture can be translated to the *in vivo* state (38). Recent studies by our group attempted to address the question of superoxide production in the type 1 diabetic kidney using a dihydroethidium (DHE) probe as a measure of *in vivo* superoxide production (14). DHE is a relatively specific fluorescent indicator of superoxide when given *in vivo* and imaged by real-time analysis or by confocal microscopy. The specific oxidized form of DHE was found to be ethidium as measured by fluorescence lifetime decay (20). Using both real-time measurements and confocal analysis, there was a surprising reduction of DHE fluorescence in glomeruli and cortical tubules, indicating reduced superoxide production in the diabetic kidney *versus* the control kidney (14). A similar reduction of superoxide was also found using electron paramagnetic resonance of freshly harvested kidney cortical tissue as compared with the control kidney. In addition, H_2O_2 generation as measured with Amplex Red was also found to be reduced from mitochondria of the diabetic kidney in response to various substrates. Thus a combination of approaches all found that superoxide generation from the kidney and ROS production from the mitochondria of the diabetic kidney were actually reduced. Nevertheless, overall production of hydrogen peroxide, nitrotyrosine, and 8-hydroxy-2′-deoxyguanosine were all increased in the kidney or urine from diabetic mice (Fig. 4). The overall conclusion was that mitochondrial oxidative activity was reduced in the diabetic kidney and may account for the reduction in superoxide production.

The basis for reduced superoxide production is unclear. It is likely that reduced mitochondrial electron transport activity and reduced glucose oxidation may contribute to this effect. Complexes I, III, and IV activity was reduced in the diabetic kidney and associated with an overall reduction of mitochondrial content and biogenesis. Similar reductions of complexes I and III activity have been found in muscle tissue of patients with diabetes (29). It has been argued that the reduction in complex I activity is due to an initial elevation of superoxide initially generated from increased electron complex activity. However, the data for this cyclical argument are not conclusive, especially from *in vivo* studies. An enhancement of DHE-detected superoxide production was associated with improved complex I and III activity and reduced renal inflammation and fibrosis when AMP-activated protein kinase (AMPK) was activated in diabetic mice (14). Furthermore, studies with DHE demonstrated an enhanced DHE/superoxide response in mitochondrial SOD2-deficient mouse kidneys without any enhanced diabetic kidney disease in SOD2-deficient mice (14). These studies do not support the contention that a primary stimulation of mitochondrial superoxide is necessary for subsequent reduction of mitochondrial ETC activity (52). Similar data and conclusions were provided in studies with diabetic neuropathy by Fernyhough's group (5, 6, 16).

Several other groups have found increased ROS in mesangial and endothelial cells; however, the source has been linked to NOXs and NOSs. In endothelial cells, the major source of O_2^- was found to be membrane-bound oxidases, which use NADH and NADPH as substrates (27). The NOX

proteins contain NADPH and FAD-binding domains in their C-terminal region. NADPH is the electron donor to molecular O_2 to form superoxide anion. In contrast to other NOX isoforms, NOX4 has been found to primarily produce H_2O_2 (90% of ROS) and relatively less superoxide (10% of ROS) (43). NOX4 in particular has been found to be consistently upregulated in diabetic kidney disease (3, 15, 24, 54, 60, 63, 64), and the cytosolic SOD (SOD1) (17, 18) appears to be the major regulator of oxidant-mediated stress in the diabetic kidney. These studies provide convincing evidence that NOXs in the cytosolic compartment are likely the major source of harmful ROS production in diabetic kidneys. Similarly, NOX4 has been consistently found to be stimulated with obesity-related kidney disease by several groups and H_2O_2 production is stimulated as a major source of ROS in obesity-related kidney disease (10, 11, 54). The role of NOX4 in adipocyte differentiation appears to be well established (22) and NOX4 deficiency may be protective against high-fat-induced adipocyte expansion (32). However, the kidney appears to require NOX4 for the inflammation associated with obesity-related kidney disease and is largely dependent on AMPK activation.

Recently, the role of NOX4 as a key factor in kidney disease associated with podocyte dysfunction was demonstrated with the development of a podocyte-specific inducible NOX4 transgenic mouse (64). With induction of NOX4 in podocytes, there was a rapid development of glomerular volume expansion, glomerular basement membrane thickening, mesangial matrix expansion, and podocyte dropout, all features consistent with the glomerular features of diabetes- and obesity-related glomerulopathy. Data consistent with these findings were reported by an independent study in podocyte-specific NOX4 knockout mice (25). Induction of diabetes in these mice led to attenuation of albuminuria, mesangial matrix expansion, and glomerular basement membrane thickening. The downstream effects of NOX4-mediated ROS effects to cause diabetic kidney and obesity-related kidney disease are beginning to be revealed. NOX4-mediated stimulation of PKC-α may contribute to many of the NOX4-dependent effects in diabetic kidney disease (56). In addition, a novel pathway where NOX4 inhibits the enzyme fumarate hydratase and leads to accumulation of fumarate has also been linked to stimulation of transforming growth factor-β (TGF-β), matrix genes, and hypoxia-inducible factor 1-α (HIF1α) (64).

There is also convincing data that NOSs are stimulated in diabetic kidney disease and that endothelial nitric oxide synthase (eNOS) overproduction of NO may combine to form peroxynitrite in association with superoxide (40, 50). The source of the superoxide may be NOS itself or superoxide generated from NOX or cytosolic xanthine oxidase, peroxisomal oxidases, or endoplasmic reticular oxidases. When NOS is uncoupled with its cofactor tetrahydrobiopterin or its substrate, arginine, there can be a massive increase in superoxide formation (36). Overproduction of NOS isoforms as a contributor to diabetic neuropathy has also been recently demonstrated (1) (Fig. 2).

Redox Balance, AMPK, Pseudohypoxia, and HIF

Oxidant production and its effects on inflammation and fibrosis are linked to reversible redox. Redox can also be described to characterize the reaction by which oxidants affect critical proteins, such as a kinase or phosphatase, and antioxidants, such as glutathione (GSH), to reduce the protein back to its original state. The redox state and oxidants also appear to be potent regulators of the master energy sensor, AMPK.

Several groups have demonstrated an important role of AMPK in several renal diseases, including obesity and diabetic kidney disease. AMPK activation has been found to be beneficial in reducing the inflammation and fibrosis noted with a high-fat diet (10, 11), type 1 diabetes (14, 15), as well as in the subtotal nephrectomy model. AMPK activation may promote reduced inflammation *via* inhibiting NFκB activation and NOX activation (15, 51, 54, 55). In addition, AMPK activation appears to have a potent ability to reduce NOX4 production and production of H_2O_2 (10, 14). With respect to fibrosis, AMPK activation reduced TGF-β-mediated fibrosis and has been recently found to inhibit high glucose and TGF-β-induced nuclear translocation of Smad4 (65). The combined regulation of AMPK/Sirtuins/PGC1α and mTOR axis has been consistently found to play central roles in obesity- and diabetes-related kidney disease and is likely to be fundamental to many chronic kidney disorders. The interaction of ROS/RNS and antioxidants to explain the regulation of the combined axis is a major challenge to further understanding of obesity and diabetic kidney disease development and pathogenesis.

AMPK has been found to be reduced in the kidney from obese and diabetic animals, although the basis for reduced AMPK is unclear. It has been well established that even small changes in AMP are sensed by AMPK and in any state wherein AMP is increased, as in starvation and ischemia, are associated with marked stimulation of AMPK. However, the regulation of AMPK during nutrient stress or caloric overfeeding is less clear. There is likely to be short-term and chronic effects on the ATP/AMP ratio, which is a potent regulator of AMPK. Liver kinase B1 (LKB1) is a potent

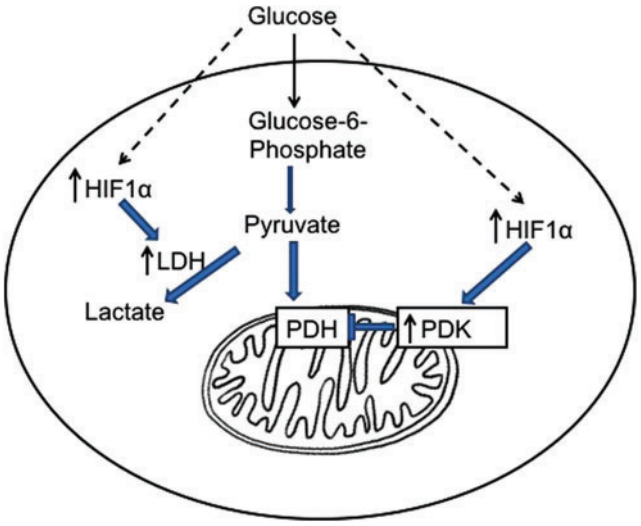

FIG. 5. HIF1α regulation and glycolysis. HIF1α is activated with high glucose and stimulates LDH to stimulate glycolysis and lactate production and inhibits PDH *via* PDK to inhibit pyruvate entry to mitochondria. HIF1α, hypoxia-inducible factor 1-α; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

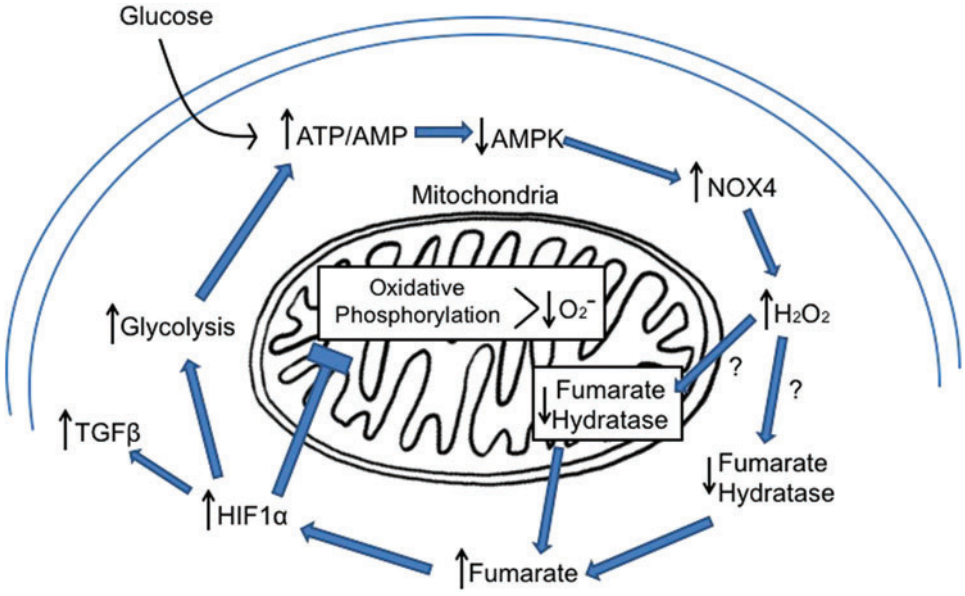

FIG. 6. Feed forward loop to maintain reduced AMPK and increase HIF1α in renal cells with increased intracellular glucose. The reduced activity of AMPK will enhance NOX4 leading to increased H_2O_2. The increased H_2O_2 reduces fumarate hydratase protein levels, leading to accumulation of fumarate. The increased fumarate can stimulate HIF1α accumulation, which drives the glycolytic process but suppresses oxidative phosphorylation and reduces mitochondrial superoxide. AMPK, AMP-activated protein kinase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

regulator of AMPK and has been found to be reduced in the diabetic kidney and may be a major basis for reduced AMPK activity in the diabetic kidney (31). Several groups have found that ROS, including H_2O_2 and superoxide, may stimulate AMPK activity and contribute to the stimulation of AMPK in ischemic and hypoxic states marked by overproduction of ROS (4). Similarly, there is convincing data that RNS, such as NO and peroxynitrite, are also potent stimulators of AMPK (4). However, there is concomitant reduction of AMPK in the diabetic state along with an overproduction of H_2O_2 and peroxynitrite. Whether there is suppression of AMPK by an excess of ATP, reduced LKB1 activity, or reduced mitochondrial superoxide production remains unclear.

Along with regulation of AMPK, there is convincing data that hypoxia and HIF1α are regulated in the diabetic kidney. Hypoxia is of course a potent stimulus for HIF1α, whereas in the diabetic state there is stimulation of HIF1α *in vivo* but no apparent reduction in overall oxygen content (Fig. 5). The stimulation of HIF1α is consistently observed in the *in vivo* state but surprisingly not replicated by high glucose in cell culture models of diabetic kidney disease. Stimulation of HIF1α would lead to stimulation of ''anaerobic'' glycolysis and lactate production *via* enhanced lactate dehydrogenase as well as suppression of mitochondrial oxidative phosphorylation and TCA activity *via* activating pyruvate dehydrogenase kinase (PDK). Enhanced activity of PDK will phosphorylate the pyruvate dehydrogenase E1a subunit, leading to impaired pyruvate conversion to acetyl CoA (30). The concept of pseudohypoxia to explain an increase in the NADH/NAD ratio with diabetes has been postulated by Williamson *et al.* (61). However, the overall increase in NADH/NAD ratio in diabetic target tissues has not been consistently reported (12), possibly because of difficulties in the accurate measurements of NADH and NAD and the lack of robust *in vivo* approaches. Often, there is coexistent stimulation of AMPK in hypoxic states as there would be a reduction in oxygen availability for oxidative phosphorylation and a consequent reduction in ATP and accumulation of AMP. However, as noted, AMPK is typically reduced in the diabetic state at the same time that HIF1α is stimulated (Fig. 6). A possible contribution to the stimulation of HIF1α may be due to accumulation of the metabolites fumarate and succinate that can compete with alpha-ketoglutarate to inhibit prolyl hydroxylase and thus reduce HIF1α ubiquitination and degradation (37, 62). As both fumarate (64) and succinate (57) have been found to accumulate in diabetic kidney, the basis of the pseudohypoxia stimulation of HIF1α may well be due to metabolite regulation (Fig. 6). A targeted metabolomics analysis in both experimental models (2, 64) and the human condition of diabetes- (35, 53, 58) and obesity-related kidney disease (13, 66, 67) will be very useful to unravel *in vivo* regulation and translation of critical and relevant pathways of renal disease progression.

Concluding Remarks

The role of ROS, RNS, and redox balance in obesity and diabetic kidney disease has been intensively investigated in the past decade and several consensus views are emerging. There appears to be a consistent finding of an elevation in many ROS/RNS molecules, such as H_2O_2 and peroxynitrite, with their sources being likely NOX4 and uncoupled NOS (eNOS). The regulation of NOX4 and eNOS uncoupling is, in part, related to reduced AMPK activity, and stimulation of AMPK activity has been consistently linked to improved outcomes. The role of superoxide has been more controversial and its link to mitochondrial function will need to be revised as additional advances in visualizing mitochondrial function *in vivo* are being developed. Redox relationships are central to obesity- and diabetes-related kidney disease and further analysis of specific regulators of redox with metabolic pathways will be central to the explanation of nutrient or caloric-stress responses in the kidney.

Acknowledgments

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Address correspondence to:
Dr. Kumar Sharma
Center for Renal Translational Medicine
Institute of Metabolomic Medicine
University of California San Diego
Stein Clinical Research Building
4th Floor, 9500 Gilman Drive
La Jolla, CA 92093-0711

E-mail: kumarsharma@ucsd.edu

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Abbreviations Used

AMPK = AMP-activated protein kinase
ATP = adenosine triphosphate
eNOS = endothelial nitric oxide synthase
ETC = electron transport chain
HIF1α = hypoxia-inducible factor 1-α
LKB1 = liver kinase B1
NOS = nitric oxide synthase
NOX = NADPH oxidase
PDK = pyruvate dehydrogenase kinase
PKC = protein kinase C
RNS = reactive nitrogen species
ROS = reactive oxygen species
TCA = tricarboxylic acid
TGF = transforming growth factor