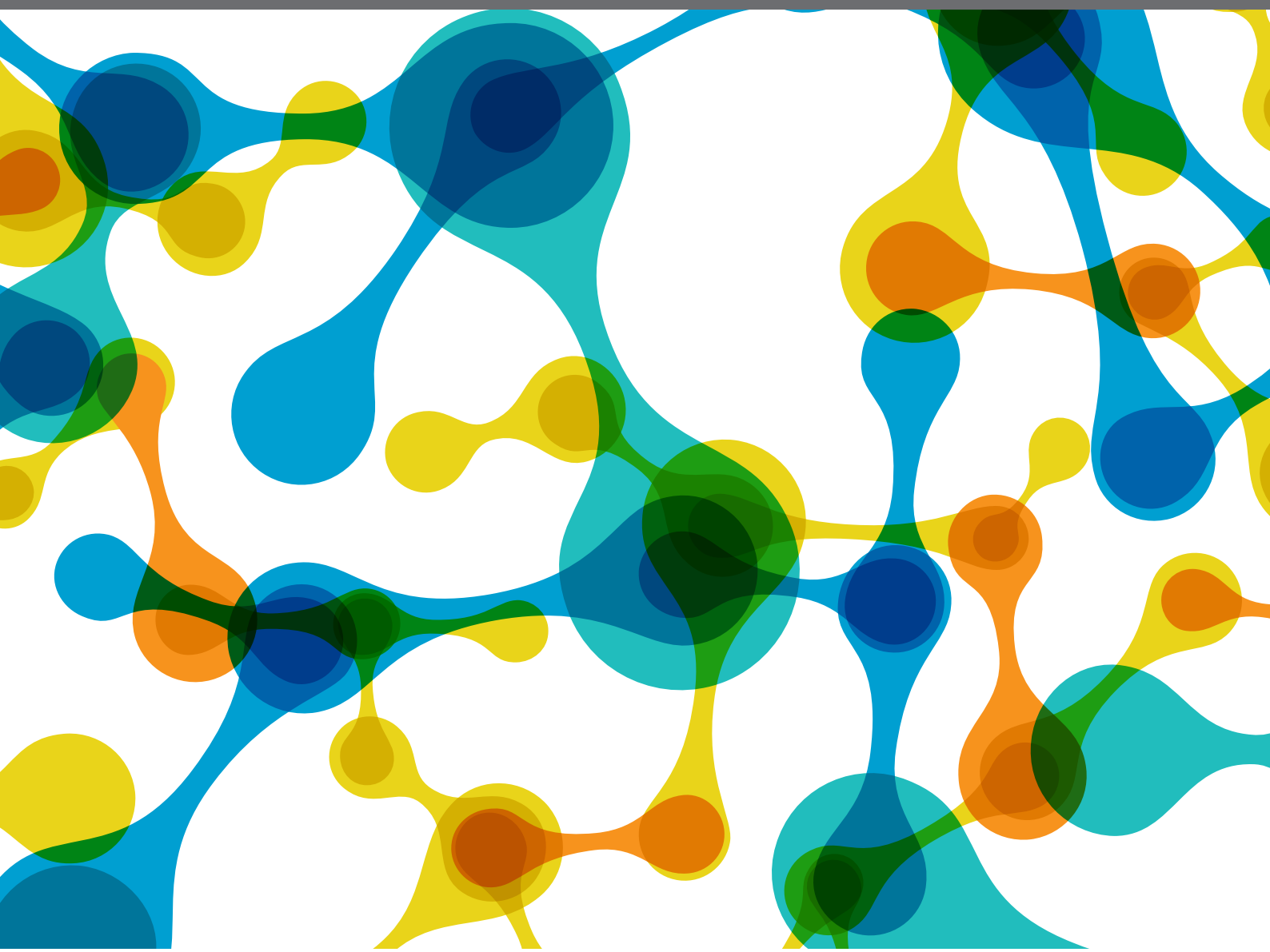
MITOCHONDRIA IN RENAL HEALTH AND DISEASE

EDITED BY: Egor Plotnikov and Giuliano Ciarimboli
PUBLISHED IN: Frontiers in Physiology and
Frontiers in Cell and Developmental Biology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-404-9
DOI 10.3389/978-2-88971-404-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MITOCHONDRIA IN RENAL HEALTH AND DISEASE

Topic Editors:
Egor Plotnikov, Lomonosov Moscow State University, Russia
Giuliano Ciarimboli, University of Münster, Germany

Citation: Plotnikov, E., Ciarimboli, G., eds. (2021). Mitochondria in Renal Health and Disease. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-404-9

Table of Contents

frontiers in Physiology

EDITORIAL
published: 30 July 2021
doi: 10.3389/fphys.2021.707175

Editorial: Mitochondria in Renal Health and Disease

Egor Plotnikov[1]* *and Giuliano Ciarimboli*[2]*

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia, [2] Experimental Nephrology, Medicine Clinic D, University of Münster, Munster, Germany

Keywords: acute kidney injury, chronic kidney disease, mitophagy, nephrotoxicity, lipotoxicity, antioxidants

Editorial on the Research Topic

Mitochondria in Renal Health and Disease

The mitochondria is a unique cell hub that integrates many signaling pathways and determines the fate of the cell. Proper functioning of the mitochondria is especially important for the kidney, since the kidney is one of the most energy-demanding organs. Energy is required to sustain the intense transport processes, which constitute one of the most important renal function. In terms of the relative rate of oxygen consumption, kidney mitochondria are in second place after the heart (Boveris et al., 2006), ahead of the liver and brain. In addition, the kidney mitochondrial abundance is higher, compared to the brain and heart, second only to the liver (Schulz et al., 2015). While the production of energy and ATP is the key function of the mitochondria in the kidney, these organelles also regulate such processes as apoptosis, steroid synthesis, calcium and iron homeostasis, and a number of others that are discussed in the Topic.

It is known that the main substrate for energy production in the renal tubules are fatty acids, so in the Research Topic several papers discuss the effect of kidney mitochondria on lipid metabolism and the relationship of disorders in β-oxidation of fatty acids and the development of various kidney pathologies. Thus, in review by Lin and Duann, the recent advances in understanding of lipid metabolism role in the function of kidney mitochondria and the molecular mechanisms related to dyslipidemia during kidney disease progression are summarized. Authors discuss molecules targeting mitochondrial lipid metabolism and dysfunction, including pharmacological agents promoting mitochondrial fatty acid oxidation, mitochondrial biogenesis, and ATP synthesis, as well as mitochondrial antioxidants and cardiolipin stabilizers.

In the review by Console et al. the network of transporters and enzymes responsible for the mitochondrial fatty acid oxidation is considered in detail, focusing on derangements that underlie acute kidney injury (AKI). Restoring mitochondrial β-oxidation might reverse or attenuate renal failure. As the PGC-1α/PPARα axis governs transcriptional regulation of fatty acid oxidation, it was proposed as therapeutic target in AKI.

The complex interaction of mitochondria and lipotoxicity is discussed in detail in a review by Ge et al. They prove that there is a vicious cycle when stress-induced reactive oxygen species (ROS) production inhibits mitochondrial fatty acid β-oxidation and lipid utilization, which causes lipotoxicity and, in turn, increased ROS-generation. Thus, authors provided the evidence that both altered mitochondrial function and lipid metabolism may contribute to the pathogenesis of kidney diseases via lipid accumulation. Therefore, identification of therapeutic targets that break this vicious cycle may offer novel therapeutic interventions.

High energy-demand is characteristic of both the kidney and the muscles that is why mitochondrial dysfunction is a common event for pathologies such as chronic kidney failure and sarcopenia. Chronic kidney disease (CKD) is an illness that by itself exhibits sarcopenia symptoms. In the review by Takemura et al. the data indicating mitochondrial dysfunction in the skeletal

OPEN ACCESS

Edited and reviewed by:
Carolyn Mary Ecelbarger, Georgetown University, United States

Correspondence:
Egor Plotnikov
plotnikov@belozersky.msu.ru
Giuliano Ciarimboli
gciari@uni-muenster.de

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *09 May 2021*
Accepted: *09 July 2021*
Published: *30 July 2021*

Citation:
Plotnikov E and Ciarimboli G (2021) Editorial: Mitochondria in Renal Health and Disease. Front. Physiol. 12:707175. doi: 10.3389/fphys.2021.707175

muscle of patients with CKD are reported. Authors discuss therapeutic strategies with some factors related to the mitochondria in CKD that could provide renoprotection.

One of the main consumers of energy produced by the kidney mitochondria are ion pumps, which provide the transport of organic compounds and inorganic ions through the tubular cells. Membrane proteins such as organic anion transporters and the organic cation transporters are highly expressed on the proximal tubular cells and carry a wide range of solutes across the plasma membrane. In addition to the natural metabolites, these carriers are able to transport a number of xenobiotics, including drugs. As a result of accumulation of such nephrotoxic agents, a drug-induced AKI can develop. The mechanisms of this accumulation of drugs and their negative impact on the functioning of mitochondria are discussed in the review by Gai et al.

In addition to the excretion of drugs and xenobiotics, the kidneys maintain the homeostasis of various metal ions, whose amount can raise excessively because of some pathologies (hemochromatosis, hemoglobinuria) or consumption with food. Mitochondria are the main buffer and storage for ions such as iron, copper, manganese, and calcium, and on the other hand, it can suffer from their toxic effects. These cross-talk between mitochondria and metal ions are discussed in the review by Thévenod et al. The authors showed that the renal mitochondria have special transporters for iron in the outer mitochondrial membrane, which are also able to transport cadmium. The accumulation of these metals in the kidney mitochondria leads to its damage and the development of nephrotoxicity and AKI.

Calcium ions, which are also buffered by the mitochondria, are important for the development of urolithiasis. The association among mitochondrial dysfunction, intracellular or mitochondrial calcium concentration, and kidney stone pathogenesis are comprehensively discussed in the article by Chaiyarit and Thongboonkerd. They point that mitochondrial dysfunction alone is not sufficient to induce kidney stone formation, however, it is associated with oxidative stress and tissue inflammation in kidney.

The majority of renal pathologies are associated with the development of oxidative stress, in which mitochondria play a key role by producing ROS. Therefore, the antioxidant systems of the renal cells play a pivotal role in stress tolerance. The review by Kitada et al. examines the key functions of the mitochondrial antioxidant enzyme MnSOD, as well as the possibilities of correcting redox homeostasis with mitochondria-targeted antioxidants.

Finally, the importance of maintaining a pool of normally functioning mitochondria in kidney cells was demonstrated in the work of Zhu et al. The authors investigated the role of autophagy mediated by PINK/Parkin in the protection of kidney cells from ischemic damage. The protein augmenter of liver regeneration (ALR), which is ubiquitously expressed, stimulated the activation of mitophagy after ischemia/reperfusion, resulting in reduced mitochondrial dysfunction and production of ROS.

CONCLUSION

Mitochondria are pivotal for maintaining the health and function of the metabolically active kidney by providing efficient energy support for maintenance of ions homeostasis and elimination of waste metabolites and environmental toxicants, including drugs. The articles collected in the Research Topic show that mitochondria are central element of several damaging cycles, such as lipotoxicity, accumulation of bivalent metal ions, and drug-induced AKI. In all these cases, protection of the renal mitochondria and effective mitochondrial quality control can be the factor that breaks the vicious circle and prevents the loss of kidney function.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

The work was supported by Russian science foundation (grant #21-75-30009 for EP).

REFERENCES

Boveris, A., Valdez, L. B., Zaobornyj, T., and Bustamante, J. (2006). Mitochondrial metabolic states regulate nitric oxide and hydrogen peroxide diffusion to the cytosol. *Biochim. Biophys. Acta* 1757, 535–542. doi: 10.1016/j.bbabio.2006.02.010

Schulz, S., Lichtmannegger, J., Schmitt, S., Leitzinger, C., Eberhagen, C., Einer, C., et al. (2015). Protocol for the parallel isolation of intact mitochondria from rat liver, kidney, heart, and brain. *Methods Mol. Biol.* 1295, 75–86. doi: 10.1007/978-1-4939-2550-6_7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 13 March 2020
doi: 10.3389/fphys.2020.00178

Augmenter of Liver Regeneration Protects Renal Tubular Epithelial Cells From Ischemia-Reperfusion Injury by Promoting PINK1/Parkin-Mediated Mitophagy

Dong-Ju Zhu[1,2], Xiao-Hui Liao[1], Wen-Qi Huang[3,4], Hang Sun[3], Ling Zhang[1] and Qi Liu[3]**

[1] Department of Nephrology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China, [2] Department of Nephrology, The Affiliated Hospital, Panzhihua University, Panzhihua, China, [3] Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China, [4] Department of Intensive Care Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

OPEN ACCESS

Edited by:
Egor Plotnikov, Lomonosov Moscow State University, Russia

Reviewed by:
Frank Park, The University of Tennessee Health Science Center, United States
Nabil Eid, United Arab Emirates University, United Arab Emirates

***Correspondence:**
Ling Zhang
zhangling@hospital.cqmu.edu.cn
Qi Liu
txzzliuqi@163.com

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *24 November 2019*
Accepted: *17 February 2020*
Published: *13 March 2020*

Citation:
Zhu D-J, Liao X-H, Huang W-Q, Sun H, Zhang L and Liu Q (2020) Augmenter of Liver Regeneration Protects Renal Tubular Epithelial Cells From Ischemia-Reperfusion Injury by Promoting PINK1/Parkin-Mediated Mitophagy. Front. Physiol. 11:178. doi: 10.3389/fphys.2020.00178

Ischemia–reperfusion (I/R) is the most common cause of acute kidney injury (AKI) and can induce apoptosis in renal epithelial tubule cells. Mitochondrial dysfunction is one of the main reasons for I/R-induced apoptosis. Accumulating evidence suggests that PINK1/Parkin-mediated mitophagy possibly plays a renoprotective role in kidney disease by removing impaired mitochondria and preserving a healthy population of mitochondria. Our previous study showed that augmenter of liver regeneration (ALR) alleviates tubular epithelial cells apoptosis in rats with AKI, although the specific mechanism remains unclear. In this study, we investigated the role of ALR in I/R-induced mitochondrial pathway of apoptosis. We knocked down ALR with short hairpin RNA lentiviral and established an I/R model in human kidney proximal tubular (HK-2) cells *in vitro*. We observed that the knockdown of ALR aggravated mitochondrial dysfunction and increased the mitochondrial reactive oxygen species (ROS) levels, leading to an increase in cell apoptosis via inhibition of mitophagy. We also found that the PINK1/Parkin pathway was activated by I/R via confocal microscopy and Western blot. Furthermore, the knockdown of ALR suppressed the activation of PINK1 and Parkin. These findings collectively indicate that ALR may protect HK-2 cells from I/R injury by promoting mitophagy, and the mechanism by which ALR regulates mitophagy seems to be related to PINK1 and Parkin. Consequently, ALR may be used as a potential therapeutic agent for AKI in the future.

Keywords: augmenter of liver regeneration, mitochondria, mitophagy, PINK1, Parkin, ischemia–reperfusion injury

INTRODUCTION

Acute kidney injury (AKI) is a common and severe renal disease characterized by a rapid loss of kidney function. Mounting studies reported that AKI is an independent risk factor for end-stage kidney disease and chronic kidney disease and that severe AKI can induce multiorgan dysfunction (Chawla et al., 2014; Ferenbach and Bonventre, 2015; Venkatachalam et al., 2015). No effective

treatment is available; thus, severe AKI is accompanied with a high mortality and has become a global public health issue and socioeconomic burden. A range of factors can lead to AKI, including ischemia reperfusion (I/R) injury, sepsis, and nephrotoxins (Mehta et al., 2016). Renal I/R is a main cause of AKI and is characterized by a reduction in the tissue blood supply, which causes acute tubular injury. Re-establishing the blood supply following prolonged ischemia stimulates vascular endothelial cells and enhances the generation of reactive oxygen species (ROS). The excessive production of ROS causes oxidative damage in renal tubular epithelial cells, ultimately leading to apoptosis or necrosis (Malek and Nematbakhsh, 2015).

Although the molecular mechanism underlying renal tubular epithelial cell apoptosis in I/R injury is still poorly understood, compelling evidence suggests that mitochondrial dysfunction plays a critical role in apoptosis by producing ROS and releasing cell death factors (Kezic et al., 2016; Yu and Lee, 2016). Cellular stresses, such as ischemia, hypoxia, and glucose deprivation, can cause mitochondrial damage or dysfunction. Therefore, the timely removal of these organelles is critical to cellular homeostasis and viability. Mitophagy, a type of selective autophagy, is a key mechanism in controlling mitochondria quality (Tatsuta and Langer, 2008; Ishihara et al., 2013; Hall and Schuh, 2016). Currently, two main mechanisms of mitophagy have been suggested (Parkin-dependent and Parkin-independent pathways). The Parkin-dependent pathway involves phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and Parkin RBR E3 ubiquitin protein ligase [Parkinson disease 2 (Parkin)], which is the most recognized mitophagy pathway in mammalian cells (Valente et al., 2004). In addition, the Parkin-independent pathway of mitophagy is mediated by Bcl-2 and adenovirus E1B 19-kDa-interacting protein 3 (BNIP3) or BNIP3-like (BNIP3L), which is critical for erythroid maturation (Ashrafi and Schwarz, 2013). Notably, a recent study reported that FUNDC1, a mitochondrial outer-membrane protein, mediates hypoxia-induced mitophagy in mammalian cells and protects the heart from I/R injury (Liu et al., 2012; Zhang et al., 2017).

Augment of liver regeneration (ALR), also known as hepatic stimulator substance, specifically stimulates the regeneration of hepatocytes (LaBrecque and Pesch, 1975). ALR protein is encoded by the growth factor erv1-like (GFER) gene and is highly homologous to the yeast ERV1 family protein (Lange et al., 2001). Accumulating evidence has shown that ALR is expressed ubiquitously in all organs, including the kidney (Tury et al., 2005; Liao et al., 2010). ALR is expressed as a short (15 kDa) and long form (23 kDa). The 15-kDa ALR is mainly secreted from hepatocytes into the extracellular environment, while the 23-kD ALR is reported to in the intermembrane space (IMS) of mitochondria and participates in a redox relay system that mediates the import of proteins to the IMS (Mordas and Tokatlidis, 2015; Nalesnik et al., 2017; Jiang et al., 2019). Increasing evidence has shown that ALR has an antiapoptotic effect (Polimeno et al., 2012). Our earlier study reported that ALR expression is markedly increased in the renal cortex or outer stripe of the outer medulla in rats with AKI (Liao et al., 2009). Intraperitoneal injection of exogenous recombinant ALR enhances the proliferation of renal tubular cells and prevents tubular cell apoptosis (Liao et al., 2010). Furthermore, we found that the knockdown of ALR suppresses mitochondrial biogenesis in I/R injury *in vitro* (Huang et al., 2018). However, if and how ALR participates in the mitophagy pathway of apoptosis in AKI remains unknown, although mitochondria deficiencies are closely associated with apoptosis.

In the present study, we used human kidney proximal tubular (HK-2) cells to establish an I/R model *in vitro* (Huang et al., 2018; Tang et al., 2018), and ALR was knocked down in HK-2 cells by infection with short hairpin RNA (shRNA) lentiviral particles to investigate the effects of ALR on cell apoptosis, mitochondrial dysfunction, and PINK1/Parkin-mediated mitophagy during I/R injury.

MATERIALS AND METHODS

Cells, Reagents, and Antibodies

HK-2 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, United States). The ALR shRNA lentiviral and control shRNA lentiviral were purchased from Genechem Co. Ltd (Shanghai, China). The JC-1 fluorescence kit and Annexin V-FITC/PI apoptosis detection kit were obtained from Sigma-Aldrich (MO, United States). MitoSOX was obtained from Invitrogen (Thermo Fisher, MA, United States). 4,6-Diamidino-2-phenylindole was acquired from KeyGen Biotech. Co. Ltd (Nanjing, China). The ALR primer was designed by Sangon Biotech Co., Ltd (Shanghai, China). Total RNA extraction kit and its reverse transcription kit were obtained from Takara Biotechnology (Shiga, Japan). Anti-PINK1, anti-Parkin, anticytochrome c oxidase subunit IV (anti-COX IV), antitranslocase of outer mitochondrial membrane 20 (anti-TOMM20), antitranslocase of inner mitochondrial membrane 23 (anti-TIMM23), anti-P62, and anticleaved caspase-3 (CASP-3) were purchased from Abcam (Cambridge, United Kingdom). Anti-ALR and anti-LC3B were purchased from Thermo Scientific (Thermo Fisher) and Cell Signaling Technology (CST, United States), respectively. Antibeta actin (anti-β-actin) was from Tianjin Sungene Biotech Co., Ltd (Tianjin, China). Cell mitochondrial extraction kits and all secondary antibodies for fluorescent labeling or immunoblot analysis were from Beyotime Biotech Co., Ltd (Shanghai, China).

Cell Culture and Lentiviral Transduction

HK-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 media (Hyclone, Logan, UT, United States) with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, United States). All cells were cultivated at 37°C in a humidified incubator with 5% CO_2. HK-2 cells were plated in 6-cm culture dishes, then transduced with LV-ALR shRNA and LV-shRNA at a multiplicity of infection (MOI) of 4, according to the manufacturer's operations. After 72 h, the successfully transduced cells were selected with puromycin (Sigma-Aldrich Corporation). For stable transduction, transduced cells were cultured in complete medium containing 3 μg/ml of puromycin

(this concentration of puromycin kills all normal HK-2 cells) for 2 weeks for the next experiment. Western blotting and a quantitative real-time polymerase chain reaction (qPCR) were performed to verify the efficiency of infection. The sequences used to clone the full-length ALR gene were as follows: forward primer, 5′-ATCGGGATCCCGCCACCATGGCGGCGC CCGGCGA-3′; and reverse primer, 5′-CGGGTACCGGTGTCA CAGGAGCCA TCCTTC -3′.

Model of Ischemia/Reperfusion Injury *in vitro*

To induce I/R injury *in vitro*, HK-2 cells initially were cultured with serum-free media for 12 h to synchronize cell growth. After that, HK-2 cells were washed twice with sterile phosphate-buffered saline (PBS) and incubated in D-glucose free and serum-deprived media in a hypoxic incubator (Thermo Fisher) with 1% O_2, 5% CO_2, and 95% N_2 for 6 h at 37°C. Then, HK-2 cells were moved to a humidified incubator containing 21% O_2 and 5% CO_2 and cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) for 3, 6, 12, or 24 h. At the end of treatment, cells were monitored morphologically or harvested with the indicated buffers to collect cell lysates for biochemical analyses.

Lactate Dehydrogenase Assay

A lactate dehydrogenase (LDH) cytotoxicity assay kit (Beyotime Biotech Co., Ltd, Shanghai, China) was used to test cell injury caused by I/R. The observation time point was 12 h of reperfusion followed by 6 h of ischemia. According to the manufacturer's recommendations, normal and infected lentiviral cells were plated at a density of 3×10^4 per well in 96-well plates. After I/R treatment, the supernatant was collected via porous plate centrifugation ($400 \times g$ for 5 min). The optical density (OD) at a wavelength of 460 nm was measured using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, United States). The level of LDH release was calculated according to the instructions. The experiment was performed three times under the same conditions.

Isolation of Mitochondria

Isolation of the mitochondria was performed with a cell mitochondrion extraction kit (Beyotime Biotech Co., Ltd) in accordance with the manufacturer's guidelines. In brief, cells were harvested after centrifugation at $500 \times g$ for 5 min, then washed with ice-cold PBS. A glass homogenizer was then used to fully homogenize cells after adding the mitochondrial separation reagent and phenylmethylsulfonyl fluoride (PMSF) from the kit. After centrifugation at $1{,}000 \times g$ for 10 min at 4°C, the supernatant was collected, transferred to an ice-cold tube, and centrifuged at $11{,}000 \times g$ for 10 min at 4°C. Subsequently, the pellet was resuspended in mitochondrial lysate containing PMSF and contained the mitochondrial proteins.

Confocal Microscopy

Confocal microscopy was used to investigate whether LC3, PINK1, or Parkin colocalized with the mitochondria. TOMM20, a mitochondrial outer membranous protein, was used to label mitochondria. HK-2 cells were seeded onto a glass-bottomed dish (NEST, Beijing, China) for confocal microscopic observation. After I/R treatment, HK-2 cells were rinsed twice with PBS and then fixed with 4% paraformaldehyde for 20 min at 37°C. All cells were permeabilized with 0.3% Trion X-100 for 20 min and washed with PBS. Next, the cells were blocked with 5% bovine serum albumin (BSA) and incubated overnight at 4°C in primary antibody (concentration of antibodies according to manufacturer's instruction). The cells were rinsed twice with PBS and then further incubated with fluorescein isothiocyanate (FITC)- or tetramethylrhodamine isothiocyanate (TRITC)-conjugated secondary antibody at room temperature for 1 h. At last, 4,6-diamidino-2-phenylindole was used to dye the nuclei. The images were detected and analyzed via laser scanning confocal microscopy (Nikon, Ti2000, Japan).

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from lentiviral infected and normal HK-2 cells using total RNA extraction kits (Takara, Kusatsu, Japan). Total RNA was reverse-transcribed to cDNA using a PrimeScript^{TM} II Reverse Transcriptase kit (Takara). Real-time PCR was performed using SYBR Premix (Takara). For quantitative analysis, the ALR gene and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a positive control were amplified by SYBR Premix using a CFX Connect Real-Time system (Bio-Rad, Hercules, CA, United States). Relative gene expression was then analyzed using $2^{-\Delta\Delta Ct}$. The human ALR and GAPDH primers were as follows, ALR: forward, 5′-CAG AAG CGG GAC ACC AAG TTT-3′; reverse, 5′-CAC ACT CCT CAC AGG GGTAA-3′. GAPDH: forward,5′-TGA CTT CAA CAG CGA CAC CCA-3′; reverse, 5′-CAC CCT GTT GCT GTA GCC AAA-3′. Three independent experiments were repeated.

Western Blotting

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Bioteke Corporation, Wuxi, China) containing protease and phosphatase inhibitors (Bioteke Corporation); then, protein lysates were prepared from cell homogenate via centrifugation according to the instructions. Protein samples were separated by 10 or 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (EMD Millipore, Bedford, MA, United States). The membranes were blocked with 5% BSA in TBST for at least 1 h and then incubated in specific primary antibodies at 4°C for at least 12 h. Subsequently, membranes were soaked in TBST containing secondary antibodies for 1 h and visualized by enhanced chemiluminescence detection. The gray values of the target bands were analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, United States).

Transmission Electron Microscopy

After I/R treatment, HK-2 cells from each group were collected and then fixed with 2.5% glutaraldehyde and embedded by

epoxy resin. The sample slice thickness was ∼50 nm. The morphologic alterations of mitochondria were detected by TEM (Hitachi, Tokyo, Japan).

Detection of Mitochondrial Membrane Potential

Mitochondrial membrane potential (MMP) was assessed using a JC-1 fluorescence dye. Normal HK-2 cells and I/R injured HK-2 cells were rinsed twice with PBS and incubated with JC-1 (300 nM) for 20 min at 37°C. Cells were washed twice with JC-1 staining buffer (1×), then incubated with complete medium for detection using a confocal microscope, or collected for measurement via flow cytometry with 485 nm excitation and 528 nm emission wavelengths.

Detection of Intracellular ATP

The intracellular ATP levels of HK-2 cells was assessed using an ATP assay kit (Beyotime Biotech, Shanghai, China), Experimental methods and procedures were operated according to the manufacturer's protocol. The ATP content of the cells was measured in nmol/mg protein.

Detection of Mitochondrial ROS

Normal HK-2 cells and I/R injured HK-2 cells were washed twice with PBS. Then, HK-2 cells were incubated in 5 μM MitoSOX for 10 min in the dark at 37°C. Cells in each group were collected separately, and the fluorescence levels of MitoSOX of each group was measured by flow cytometry with 488 nm excitation and 525 nm emission wavelengths.

Measurement of Apoptosis

Apoptosis was measured by immunoblot analysis of cleaved caspase-3 and flow cytometry. The process of immunoblot analysis of cleaved CASP-3 was described under Western blotting. Flow cytometry was carried out by double-labeling of Annexin V and propidine iodide following the manufacturer's instructions. Three independent experiments were repeated.

Statistical Analyses

All data are represented as mean ± standard deviation (SD). Statistical analysis between two groups was performed with Student's *t* test. Statistical analysis between multiple groups was performed with one-way ANOVA followed by Tukey's *post hoc* tests. Significant difference was considered as $p < 0.05$.

RESULTS

Mitochondrial Damage, Mitophagy, and Activation of ALR Induced in HK-2 Cells by I/R

In the present study, immunoblot analysis showed that the protein level of LC3-II, an autophagy biomarker, was significantly upregulated after reperfusion, peaking at 12 h, followed by a decrease. The change in LC3-II was associated with a gradual reduction in the mitochondrial inner membrane protein TIMM23 and translocase of outer mitochondrial membrane 20 (TOMM20). These results indicated that mitochondria clearance or mitophagy was induced in HK-2 cells subjected to I/R and was more notable after 6 h of ischemia and 12 h of reperfusion (I6R12; **Figures 1A,B**). Thus, I6R12 was selected as an observation point to perform the next experiments. Then, we used TEM to examine the changes of mitochondrial morphology and mitophagosome in HK-2 cells. After I/R treatment, mitochondrial swelling, loss of mitochondrial cristae, and vacuole formation in the mitochondria matrix were apparent in HK-2 cells (**Figure 1C-ii**), and mitophagosomes, as well as autophagosomes, were also found (**Figure 1C-iii**). There were more autophagosomes in the I/R group (**Figure 1D**). These findings further confirmed the activation of mitochondrial damage and mitophagy were induced by I/R.

Because 23-kD ALR is mainly localized in the IMS of mitochondria, and our previous study showed that 23-kD ALR had a protective role in mitochondria via reduction in ROS (Xia et al., 2015). We investigated whether 23-kD ALR was involved in I/R-induced mitophagy. We found that 23-kD ALR protein expression gradually increased at 12 h and decreased after 24 h. These changes in 23-kD ALR protein expression indicated that 23-kD ALR might be involved in the process of mitophagy induced by I/R.

PINK1/Parkin Pathway Was Activated by I/R in HK-2 Cells

In kidney disease, the PINK1/Parkin pathway was reported to play a dominant role in mitophagy (Lin et al., 2019). In this study, an activation of the PINK1/Parkin pathway at the time point of I6R12 was measured using immunofluorescence and immunoblot analysis. Immunofluorescence showed many clear green dots as a sign of PINK1 or Parkin in HK-2 cells after I/R. In contrast, only weak green immunofluorescence was observed in the normal group. Costaining with TOMM20, a mitochondrial marker, showed colocalization of PINK1/Parkin with mitochondria in I/R-treated HK-2 cells, indicating the activation of the PINK1/Parkin pathway induced by I/R. Consistent with immunofluorescence, immunoblot analysis showed that PINK1/Parkin from mitochondria was upregulated by I/R (**Figure 2**).

Silencing of ALR Increases HK-2 Cells Susceptibility to I/R Treatment

HK-2 cells were transduced with a lentiviral vector carrying ALR shRNA to knock down the expression of ALR. The expression of ALR was examined by qPCR and immunoblotting analysis. The results of immunoblotting analysis showed that the ALR protein expression in the shRNA/ALR group was significantly lower than the normal or shRNA/control group (0.29 ± 0.07, 0.55 ± 0.01, 0.53 ± 0.03, $p < 0.01$; **Figures 3A,B**). Similar results were obtained by qPCR. These results demonstrate the transduction efficiency of HK-2 cells. LDH is a stable cytoplasmic enzyme in all cells and is released rapidly following plasma membrane damage and thus used

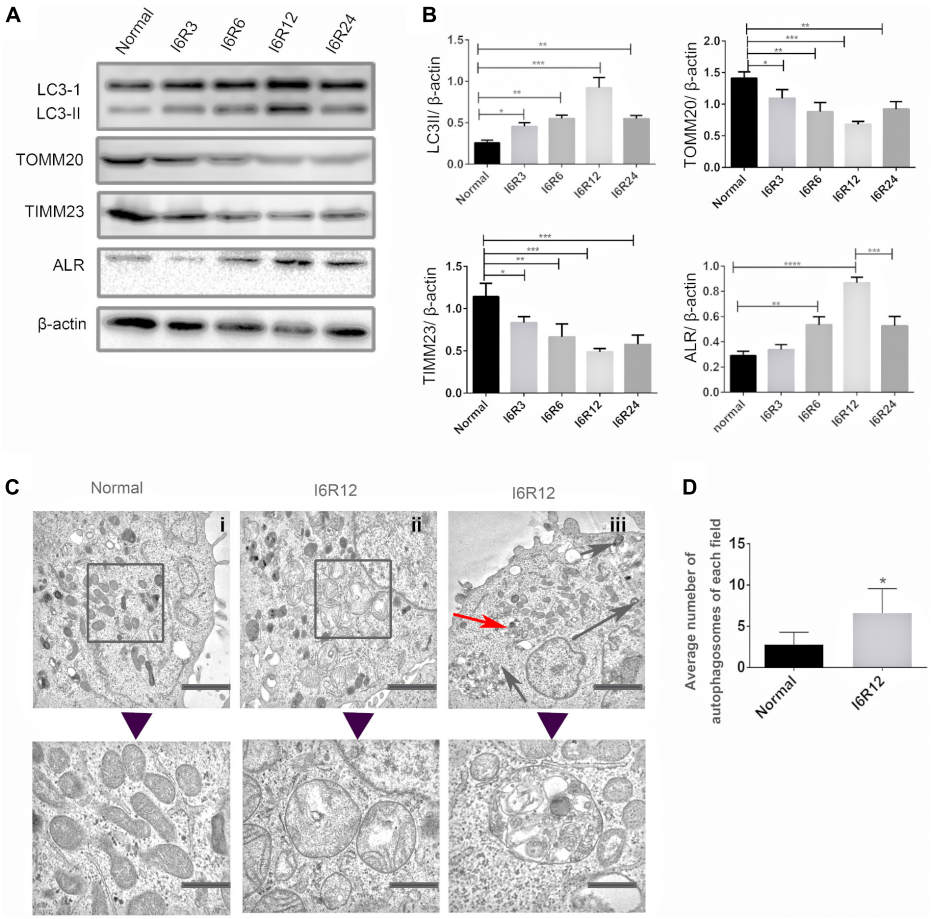


FIGURE 1 | Mitochondrial damage, mitophagy, and activation of augmenter of liver regeneration (ALR) were induced in HK-2 cells by ischemia–reperfusion (I/R). **(A,B)** Immunoblot analysis and quantification of ALR, LC3, translocase of outer mitochondrial membrane 20 (TOMM20), and translocase of inner mitochondrial membrane 23 (TIMM23). **(C,D)** Representative TEM images of mitochondrial morphology (square box), mitophagosomes (red arrow), and autophagosomes (black arrow) in HK-2 cells. The observation timepoint was I6R12. Data are shown as a bar graph of the number of autophagosomes from five images of each group. Scale bar: 2 μm. The images in the lower row show the amplification of the normal mitochondria, damaged mitochondria, and mitophagosomes. Scale bar: 200 nm. Data represent means ± SD. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

as an indicator of cell injury (Abdel-Latif et al., 2006). To investigate whether ALR has a protective effect for HK-2 cells in I/R treatment, we determined the levels of LDH release in all three groups. The results showed that the release of LDH in the shRNA/ALR group was clearly increased at the timepoint of I6R12 (**Figure 3D**). In addition, the number of cells in the shRNA/ALR group was significantly decreased after I/R compared with the shRNA/control or I/R group ($1.33 \times 10^4 \pm 0.22$, $2.15 \times 10^4 \pm 0.26$, $2.2 \times 10^4 \pm 0.18$, $p < 0.01$; **Figure 3E**). These results indicated that knockdown of ALR enhanced I/R injury in HK-2 cells.

Silencing of ALR Inhibited I/R-Induced Mitophagy in HK-2 Cells

To investigate whether ALR protects HK-2 cells from I/R injury by regulating mitophagy, we examined the occurrence of mitophagy in HK-2 cells. Confocal microscopy revealed that the shRNA/ALR group had very few green puncta of the autophagosome marker, LC3, but a large number of green puncta was shown in the shRNA/control group. Costaining with mitochondria revealed a reduction in colocalized autophagosomes and mitochondria in the shRNA/ALR group based on the Pearson's correlation coefficient to evaluate the overlap of LC3 and TOMM20. These results showed that the formation of mitophagosomes was reduced after silencing ALR (**Figures 4A,B**). Consistent with immunofluorescence, immunoblot analysis of P62, LC3-II, TIMM23, and TOMM20 revealed decreased autophagy activation and mitochondrial clearance (**Figures 4C,D**).

Silencing of ALR Inhibited the Activation of the PINK1/Parkin Pathway in HK-2 Cells During I/R

Our previous results showed that silencing of ALR inhibited I/R-induced mitophagy in our experimental model. We also

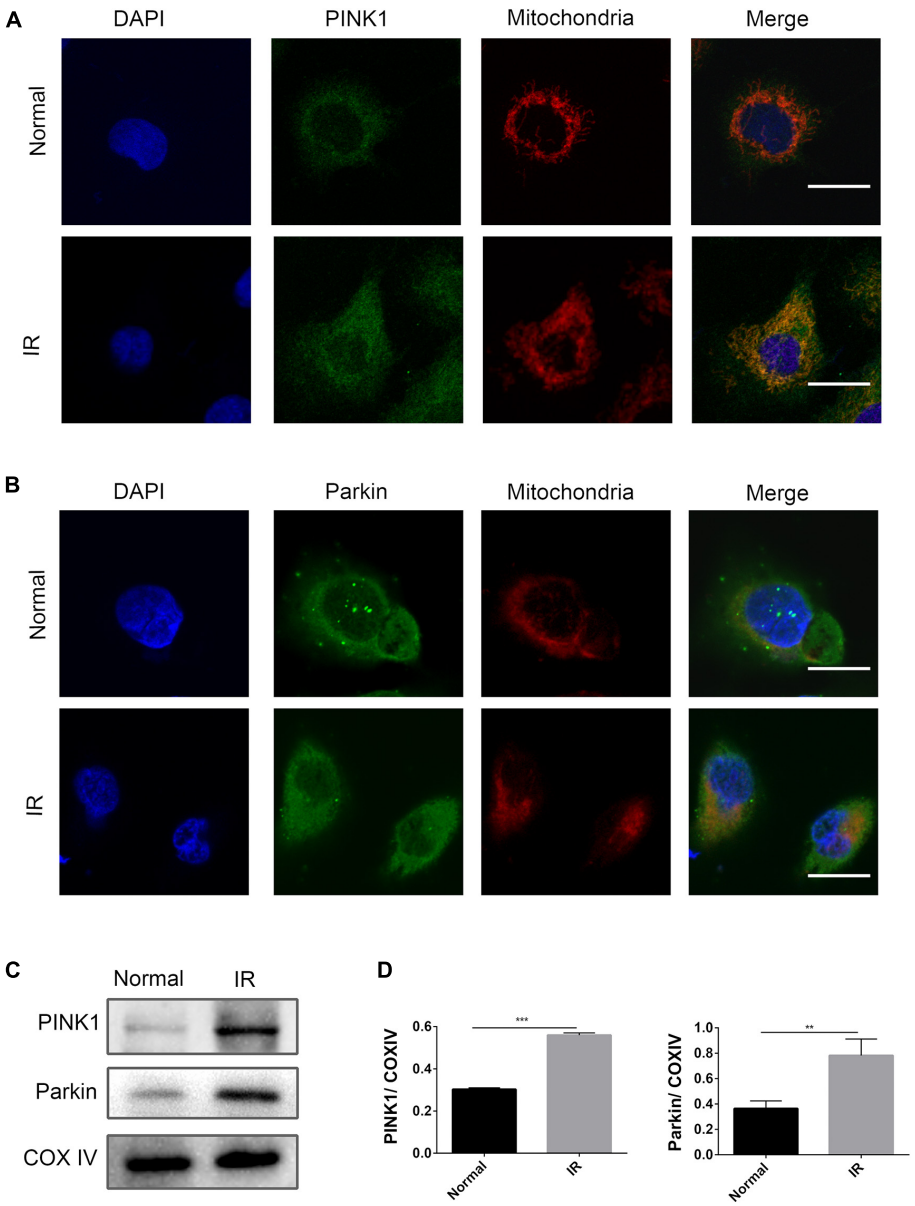


FIGURE 2 | PINK1/Parkin pathway was activated by ischemia–reperfusion (I/R) in HK-2 cells. (**A**,**B**) Representative images of immunofluorescence double-labeling PINK1/Parkin and translocase of outer mitochondrial membrane 20 (TOMM20). TOMM20 was used as a mitochondrial tracker. PINK1 or Parkin was labeled by fluorescein isothiocyanate (FITC) (green), TOMM20 was labeled by tetramethylrhodamine isothiocyanate (TRITC) (red). Strong colocalization signals in I/R group were seen as yellow dots after merging, suggesting that PINK1 or Parkin gathered on the mitochondria. Scale bar = 50 μm. **(C,D)** Immunoblot analysis and quantification of PINK1 and Parkin. Data represent means ± SD. $n = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$.

found that PINK1 and Parkin was activated by I/R, which could be critical regulatory factors in mitophagy control. Therefore, we further investigated the possibility that ALR might mediate I/R-induced mitophagy via the PINK1/Parkin pathway *in vitro*. The results showed that, in response to I/R, HK-2 cells with ALR shRNA had a decrease in PINK1 and Parkin protein expression, as well as colocalization with mitochondria in immunofluorescence staining compared with cells with control shRNA (**Figure 5**). Consequently, the signaling pathway of ALR regulating mitophagy may involve PINK1 and Parkin.

Silencing of ALR Aggravated I/R-Induced Mitochondrial Dysfunction in HK-2 Cells

Because ALR may regulate mitophagy, we further investigated the effects of ALR on I/R-induced mitochondrial dysfunction in HK-2 cells. As shown in **Figures 6A–C**, a loss of mitochondrial

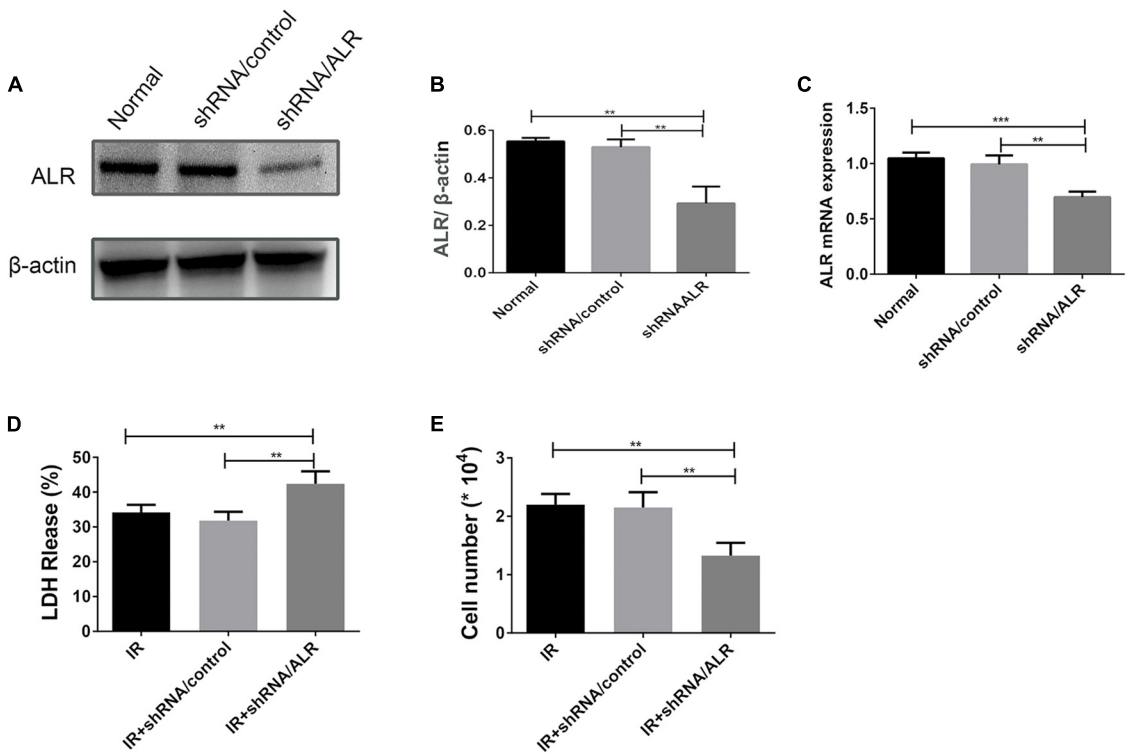


FIGURE 3 | Silencing of augmenter of liver regeneration (ALR) increases HK-2 cells Susceptibility to ischemia–reperfusion (I/R) treatment. **(A,B)** Immunoblot analysis and quantification of ALR. **(C)** The expression of ALR messenger RNA (mRNA) was measured via quantitative real-time PCR (qPCR). Data represent means ± SD. $n = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$. **(D)** The lactate dehydrogenase (LDH) released in the supernatant of cultured cells was measured at the indicated time. A significant increase was observed in the short hairpin (shRNA)/ALR group when compared with the shRNA/control group or I/R group. **(E)** The cell number was counted in three groups at I6R12. The cell number of shRNA/ALR group clearly decreased after I/R. Data represent means ± SD. $n = 4$. $^{**}p < 0.01$.

membrane potential was noted under I/R treatment, which was assessed by JC-1 staining and flow cytometry, and was further accelerated by the knockdown of ALR. In addition, silencing of ALR aggravated the inhibitory effect of I/R on ATP production (**Figure 6D**).

Silencing of ALR Enhanced I/R-Induced Mitochondrial ROS Generation

Mitochondrial dysfunction induces harm via the induction of mitochondrial ROS production or mitochondrial permeability transition, or both. The knockdown of ALR aggravated mitochondrial dysfunction. Subsequently, we explored the effect of silencing the *ALR* gene on I/R-induced mitochondrial ROS generation; we used MitoSOX kit to measure the production of mitochondrial ROS by flow cytometry. The results showed that mitochondrial ROS levels were significantly increased in the shRNA/ALR group after I/R, compared with shRNA/control group ($1.209 \times 10^6 \pm 17{,}738$, $0.898 \times 10^6 \pm 45{,}752$, $p < 0.0001$; **Figure 7**).

Silencing of ALR-Sensitized HK-2 Cells to I/R-Induced Apoptosis

To determine the effect of silencing of the *ALR* gene on I/R-induced renal tubular cell injury, we initially investigated I/R-induced apoptosis of HK-2 cells via flow cytometry. Cell apoptosis in the shALR/ALR group was higher than that of shALR/control group ($35.71 \pm 2.157\%$, $14.35 \pm 1.105\%$, $p < 0.0001$; **Figures 8A,B**). The flow cytometer assay results were confirmed by Western blot analysis of activated CASP-3. In response to I/R, the level of activated CASP-3 in HK-2 cells with silencing *ALR* was significantly higher than that of the short hairpin RNA (shRNA)/control (1.05 ± 0.14, 0.72 ± 0.05, $p < 0.01$; **Figures 8C,D**). Collectively, these findings suggested that ALR played a cytoprotective role in I/R injury *in vitro*.

DISCUSSION

The main pathological phenotype of AKI is tubular damage, including apoptosis. ALR has been reported to have an antiapoptosis function (Polimeno et al., 2012); however, the mechanism underlying this effect remains unclear. In this study, we first showed that activation of ALR and mitochondrial dysfunction were induced in I/R-AKI *in vitro*. Then, we showed that a deficiency in ALR increased mitochondrial ROS production and mitochondrial deficiencies accompanied with inhibition of PINK1/Parkin-mediated mitophagy, contributing to cell apoptosis in I/R *in vitro*. Therefore, we proposed that

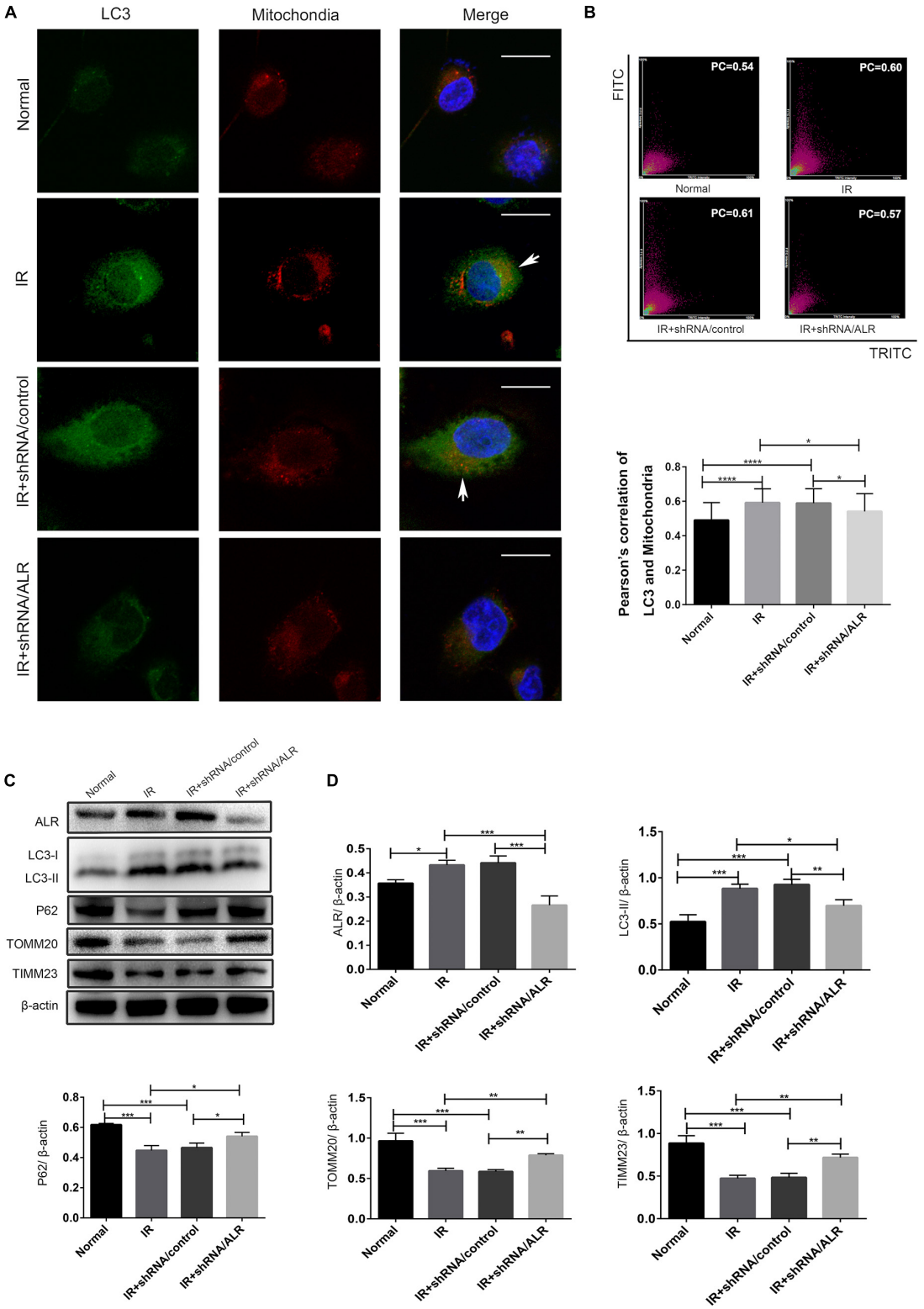


FIGURE 4 | Silencing of augmenter of liver regeneration (ALR) inhibited ischemia–reperfusion (I/R)-induced mitophagy in HK-2 cells. **(A,B)** Representative images and quantification of immunofluorescence double-labeling LC3B and translocase of outer mitochondrial membrane 20 (TOMM20) in different groups. TOMM20-labeled mitochondria (red) and LC3-labeled autophagosome (green). Scale bar: 50 μm. the Pearson's correlation columns were shown in bar graph format. Sixty cells from three independent experiments were analyzed. **(C,D)** Immunoblot analysis and quantification of ALR, LC3, P62, TOMM20, and translocase of inner mitochondrial membrane 23 (TIMM23). Data represent the means ± SD. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

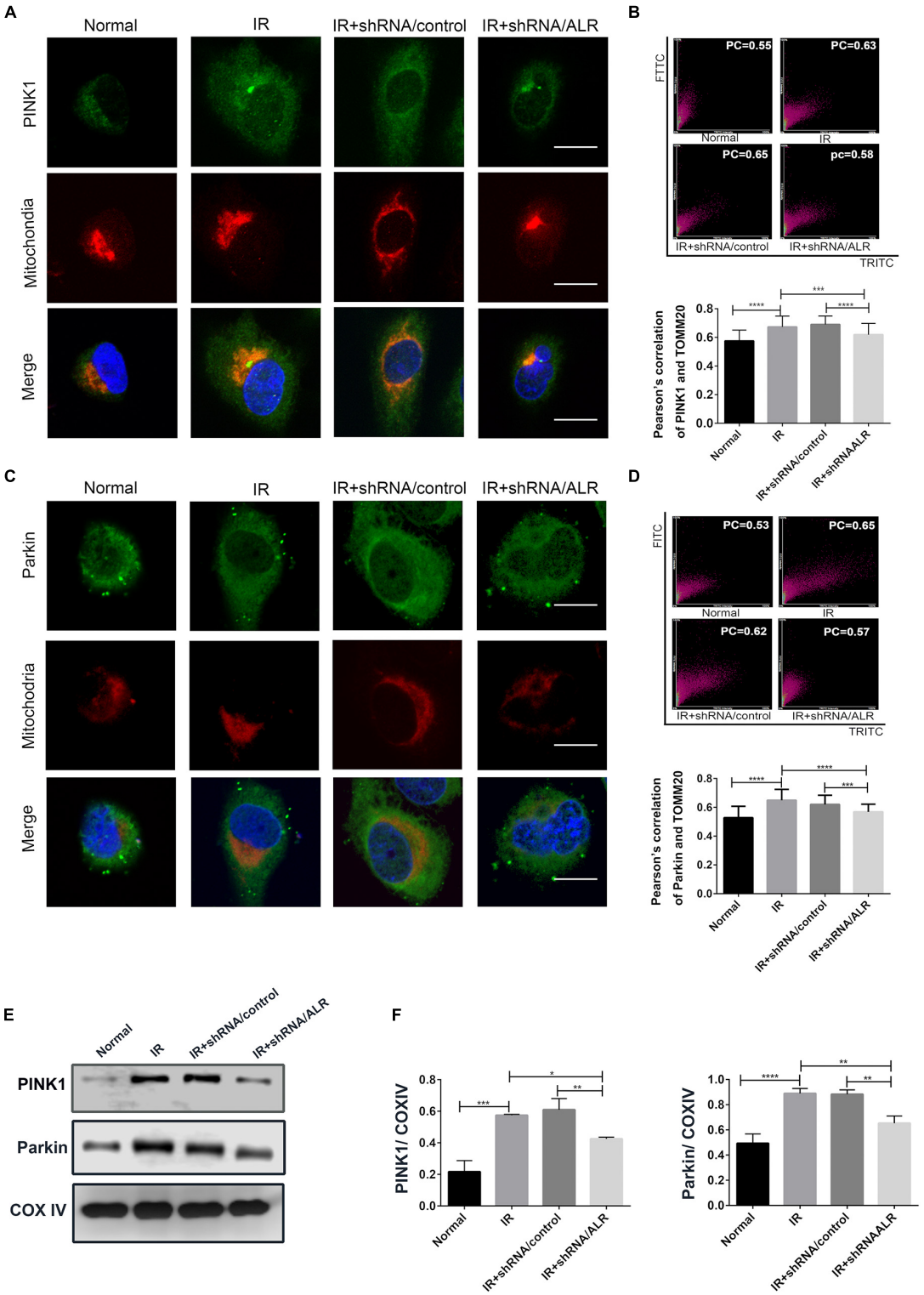


FIGURE 5 | Silencing of augmenter of liver regeneration (ALR) inhibited the activation of PINK1/Parkin pathway in HK-2 cells during ischemia–reperfusion (I/R). **(A–D)** Representative images and quantification of immunofluorescence double-labeling PINK1/Parkin and mitochondrial marker [translocase of outer mitochondrial membrane 20 (TOMM20)]. PINK1 or Parkin was labeled with fluorescein isothiocyanate (FITC) (green), mitochondria was labeled with tetramethylrhodamine isothiocyanate (TRITC) (red). Scale bar = 50 μm. the Pearson's correlation columns were shown in bar graph format. Sixty cells from three independent experiments were analyzed. **(E,F)** Immunoblot analysis and quantification of PINK1 and Parkin. Data represent means ± SD. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

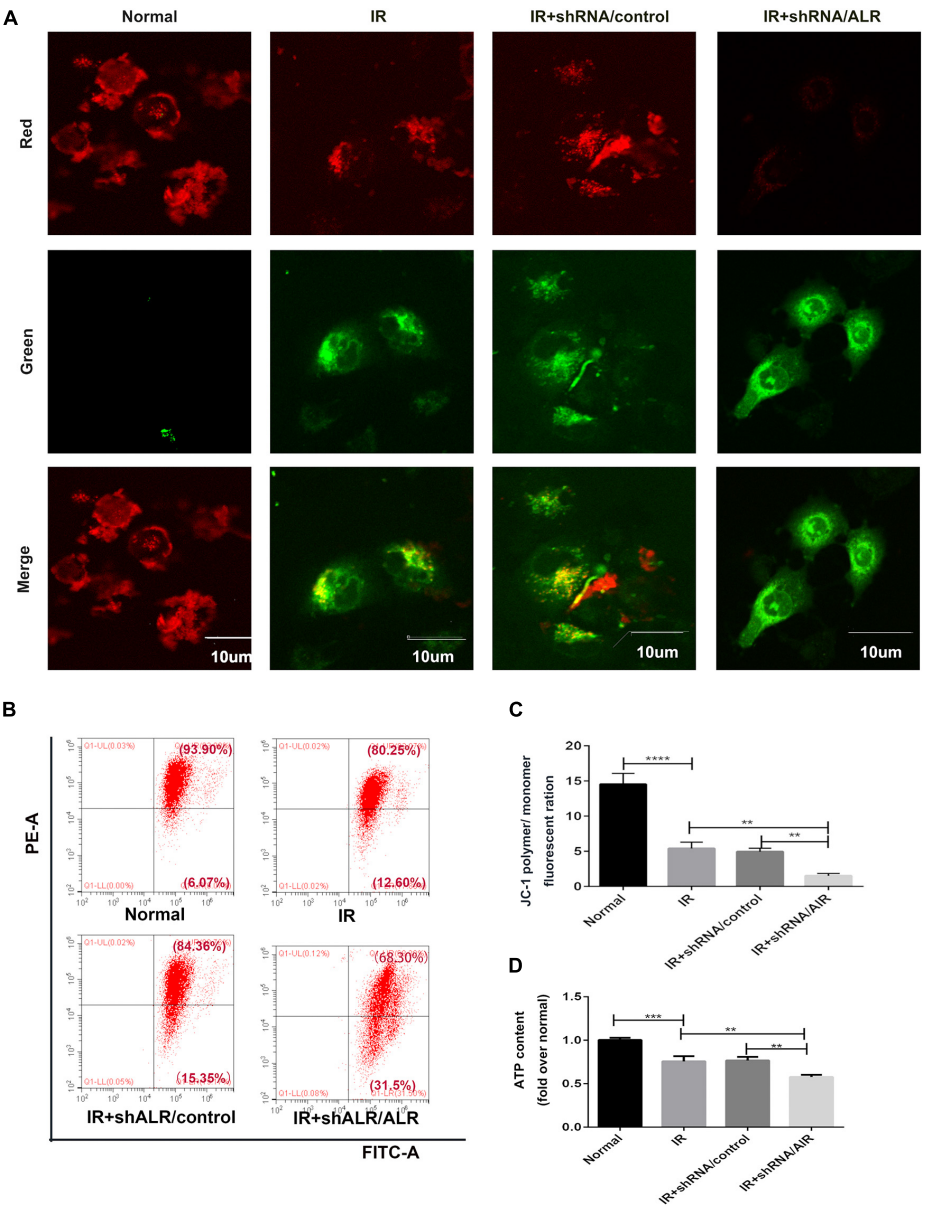


FIGURE 6 | Silencing of augmenter of liver regeneration (ALR) aggravated ischemia–reperfusion (I/R)-induced mitochondrial dysfunction in HK-2 cells. **(A)** Representative fluorescence images of HK-2 cells stained by JC-1. The picture was detected by confocal laser scanning microscopy (CLSM). Scale bar = 10 μm. **(B,C)** Representative images and analysis of mitochondrial membrane potential (MMP) via flow cytometry. **(D)** ATP contents. Using an ATP assay kit to measure ATP levels for each group. Data represent as the means ± SD. $n = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

ALR may protect renal tubular epithelial cells from I/R injury by promoting Pink1/Parkin-mediated mitophagy.

Mitochondria are the center of energy metabolism in the cell and believed to be the primary subcellular target of I/R injury. Evidence of mitochondrial dysfunction involved in human AKI has been reported in an electron microscopy study of kidney specimens from patients who had died from septic shock (Emma et al., 2016). Pathological alterations in mitochondrial structure and function have been observed in renal tubular cells in various experimental models of AKI (Parikh et al., 2015; Kezic et al., 2016; Yu and Lee, 2016). In this study, we established an *in vitro* I/R model with glucose-free culture medium and hypoxia reoxygenation according to previous studies (Jiang et al., 2010; Dong et al., 2015; Zhang et al., 2015; Huang et al., 2018). The changes in LC3-II during I/R was associated with a marked reduction in TIMM23 and TOMM20, indicating activation of mitochondrial clearance. TEM also showed damaged morphological changes in mitochondria in HK-2 cells during I/R. Aggravation of mitochondrial dysfunction

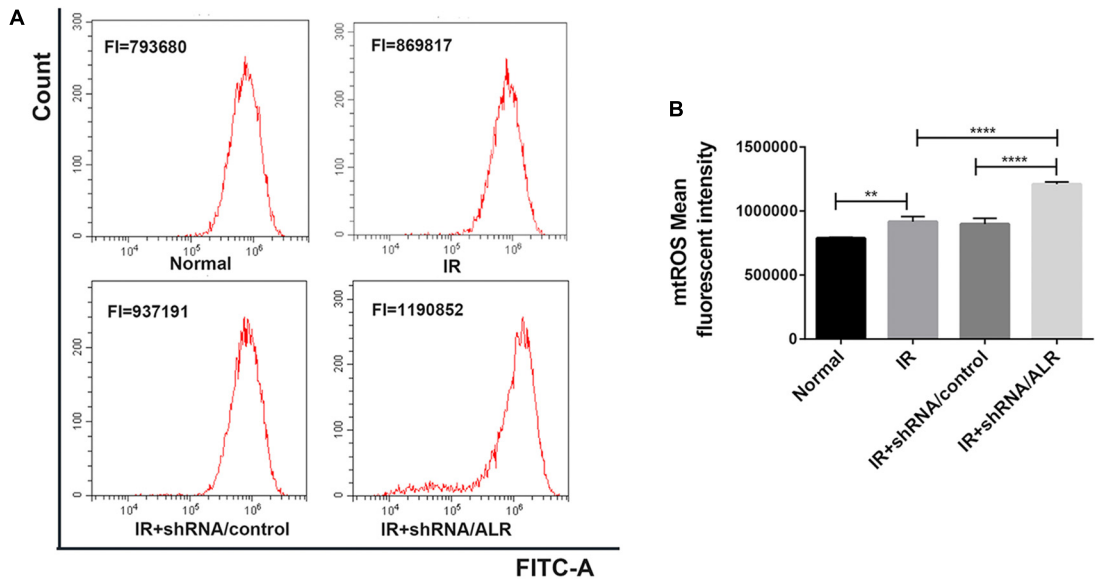


FIGURE 7 | Silencing of augmenter of liver regeneration (ALR) enhanced ischemia–reperfusion (I/R)-induced mitochondrial reactive oxygen species (ROS) generation. **(A)** The levels of mitochondrial ROS were determined by flow cytometry. **(B)** Quantitation of MitoSOX were shown in a bar graph. Data represent means ± SD. $n = 3$. $^{**}p < 0.01$, $^{****}p < 0.0001$.

was shown in HK-2 cells, as evidenced by a decrease in ATP production and mitochondrial membrane potential, which led to an increase in mitochondrial ROS and cell apoptosis. These results suggested that mitochondria damage or mitochondrial dysfunction is involved in the pathogenesis of I/R-AKI *in vitro* and closely associated with cell injury. Our previous study reported that ALR has an antiapoptotic effect in I/R injury (Liao et al., 2010). In this I/R model, we explored the link between ALR and I/R-induced mitochondrial dysfunction. In the process of I/R, ALR expression was also significantly upregulated with a time-dependent pattern, peaking at 12 h. Knockdown of ALR aggravated I/R-induced mitochondrial dysfunction, mitochondrial ROS, and cell apoptosis compared with the shRNA/control group. These observations indicated that ALR may take part in the process of I/R-induced mitochondrial dysfunction and protect cells from I/R injury. However, the underlying mechanism needs further study.

Mitophagy plays an important role in the clearance of damaged mitochondria (Linkermann et al., 2014). The reduction of mitophagy significantly exacerbated cell apoptosis and tissue damage of AKI (Zhao et al., 2017; Tang et al., 2018; Yang et al., 2018). In the present study, activation of mitophagy was found in HK-2 cells after I/R treatment. After silencing ALR, a reduction in mitophagy accompanied with an increase in mitochondrial dysfunction, mitochondria ROS, and cell apoptosis also occurred during I/R. Our research confirmed that mitophagy has a protective effect in AKI. Accordingly, ALR may play a protective role in I/R injury and mitochondria function via promoting mitophagy. It is noteworthy that the interaction between mitophagy and cell death is complex (Marino et al., 2014); emerging evidence also suggests that mitophagy is a possible mechanism for cell death programs in chronic obstructive pulmonary disease (COPD) (Mizumura et al., 2014). Therefore, the effect of mitophagy in different pathological conditions needs further investigation.

Many studies have reported that PINK1/Parkin signaling controls the selective degradation of dysfunctional mitochondria by mitophagy in acute kidney disease (Zhao et al., 2017; Tang et al., 2018; Lin et al., 2019). In healthy mitochondria, PINK1 is constitutively inserted into the mitochondria inner membrane and rapidly degraded by proteolysis to keep a low level of expression. When mitochondria are damaged, PINK1 accumulates on the mitochondrial outer membrane (MOM), which then recruits Parkin from the cytosol and phosphorylates its ubiquitin ligase. After phosphorylation, Parkin ubiquitinates various proteins in the MOM and delivers ubiquitin mitochondria to autophagosomes via combing autophagy protein P62 and microtubule-associated proteins 1 light chain 3 beta (MAP1LC3B/LC3B), inducing mitophagy and removal of damaged mitochondria (Geisler et al., 2010). In this study, we first verified the PINK1/Parkin pathway in I/R injury. Next, we found that silencing of ALR suppressed the activation of PINK1/Parkin pathway via double immunofluorescent staining and Western blot. These results suggested that ALR may regulate mitophagy via the PINK1/Parkin pathway. Furthermore, it seems that multiple molecules, such as BNIP3 and FUNDC1, are involved in mitophagy. Additional studies are needed to elucidate the exact mechanisms through which ALR exerts its regulatory function on mitophagy.

Interestingly, mounting evidence has suggested that P62 may be crucial for PINK1/Parkin-mediated mitophagy (Geisler et al., 2010). However, another study found that mitochondrial removal has taken place independently of P62 (Narendra et al., 2010).

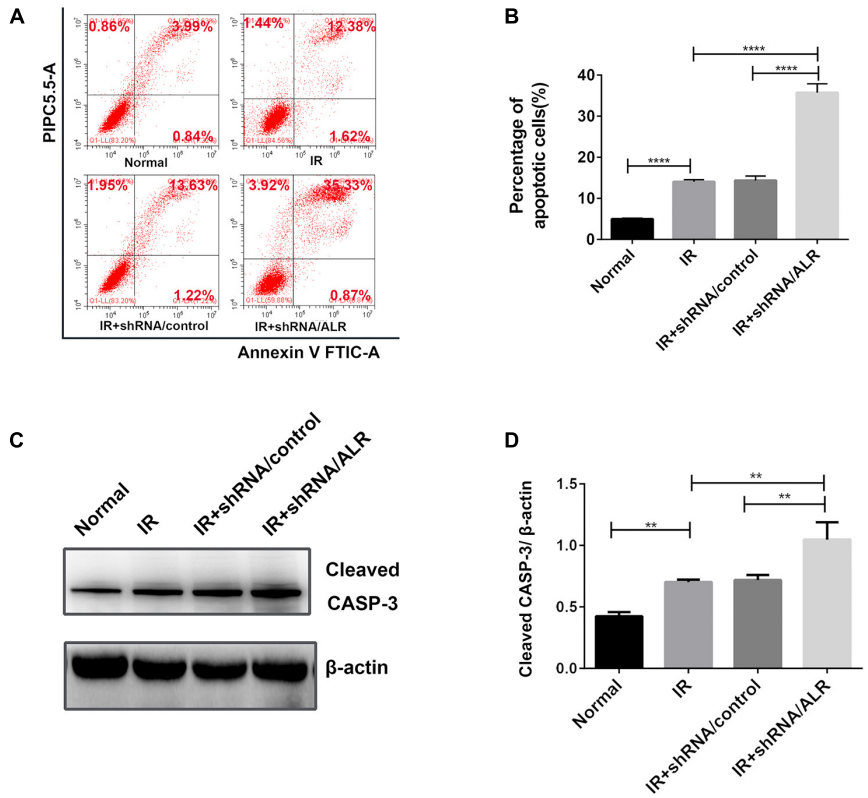


FIGURE 8 | Silencing of augmenter of liver regeneration (ALR)-sensitized HK-2 cells to induced apoptosis. Apoptosis was determined by flow cytometry and activated caspase-3 (CASP-3). **(A,B)** Representative plot and quantitation analysis of apoptosis measured by flow cytometry. **(C,D)** Immunoblot analysis and quantification of activated CASP-3. Data represent means ± SD. $n = 3$. $^{**}p < 0.01$, $^{****}p < 0.0001$.

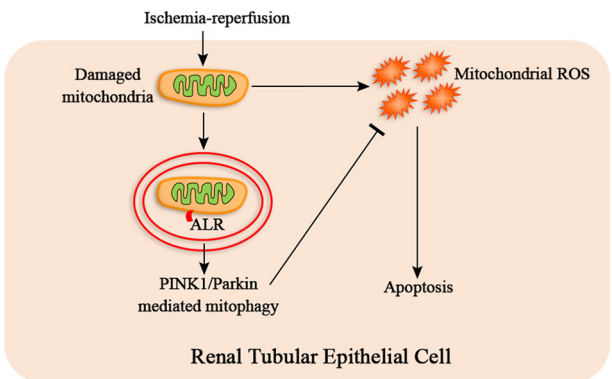


FIGURE 9 | The hypothetical mechanism of protective effect of augmenter of liver regeneration (ALR) in ischemia–reperfusion (I/R)–acute kidney injury (AKI) *in vitro*. I/R causes the mitochondrial damage of renal tubular epithelial cells, which induces mitochondrial reactive oxygen species (ROS) and apoptosis. ALR may protect renal tubular epithelial cells from apoptosis via PINK1/Parkin-mediated mitophagy, which remove the damaged mitochondria and reduce mitochondrial ROS production.

A recent study showed that instead of P62, optineurin was recruited to damaged mitochondria and transferred damaged mitochondrial to the autophagosome in PINK1/Parkin-mediated mitophagy via its LC3 interaction region domain (Wong and Holzbaur, 2014). In the present study, our results provided support that P62 may be essential in PINK1/Parkin-induced mitophagy, while the expression of P62 changed with LC3, which was consistent with the level of autophagy. Hence, the exact processes of PINK1/Parkin-mediated mitophagy need further investigation.

In summary, our research provides evidence that ALR attenuates I/R-induced mitochondrial pathway of apoptosis by reducing mitochondrial ROS production and mitochondrial dysfunction. The mechanism of protective effect of ALR may be closely associated with promoting PINK1/Parkin-mediate mitophagy (**Figure 9**). Consequently, ALR may serve as a new therapeutic target for AKI in the future.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

This experiment is an *in vitro* experiment without any ethics problems.

AUTHOR CONTRIBUTIONS

D-JZ performed the experiments and wrote the manuscript. HS performed, measured, and analyzed the confocal laser-scanning microscopy experiments. X-HL and W-QH revised and corrected the manuscript. LZ and QL designed the study and helped in analyzing and interpretation of data. All authors read and approved the manuscript.

FUNDING

This work was supported by Grants from the National Natural Science Foundation of China (81873604, 81000299, and 30971364), Grants from Chongqing Overseas Students Entrepreneurship Innovation Support Program (cx2018038), the Natural Science Foundation Project of CQ CSTC (cstc2019jscx-msxm0157 and cstc2015jcyjA10069), and The Fourth Batch of Chongqing Young and Middle-Aged Medical High-end Reserve Talent Fund [(2018) 230].

ACKNOWLEDGMENTS

We would like to thank Xian-Li Long for her support and technical assistance in the lab.

REFERENCES

Abdel-Latif, L., Murray, B. K., Renberg, R. L., O'Neill, K. L., Porter, H., Jensen, J. B., et al. (2006). Cell death in bovine parvovirus-infected embryonic bovine tracheal cells is mediated by necrosis rather than apoptosis. *J. Gen. Virol.* 87, 2539–2548. doi: 10.1099/vir.0.81915-0

Ashrafi, G., and Schwarz, T. L. (2013). The pathways of mitophagy for quality control and clearance of mitochondria. *Cell. Death. Differ.* 20, 31–42. doi: 10.1038/cdd.2012.8114

Chawla, L. S., Eggers, P. W., Star, R. A., and Kimmel, P. L. (2014). Acute kidney injury and chronic kidney disease asinterconnected syndromes. *N. Engl. J. Med.* 371, 58–66. doi: 10.1056/NEJMra1214243

Dong, W., Wang, H., Shahzad, K., Bock, F., Al-Dabet, M. M., Ranjan, S., et al. (2015). Activated protein C ameliorates renal ischemia-reperfusion injury by restricting Y-box binding protein-1 ubiquitination. *J. Am. Soc. Nephrol.* 26, 2789–2799. doi: 10.1681/ASN.2014080846

Emma, F., Montini, G., Parikh, S. M., and Salviati, L. (2016). Mitochondrial dysfunction in inherited renal disease and acute kidney injury. *Nat. Rev. Nephrol.* 12, 267–280. doi: 10.1038/nrneph.2015.214

Ferenbach, D. A., and Bonventre, J. V. (2015). Mechanisms of maladaptive repair after AKI leading to acceleratedkidney ageing and CKD. *Nat. Rev. Nephrol.* 11, 264–276. doi: 10.1038/nrneph.2015.3

Geisler, S., Holmström, K. M., Skujat, D., Fiesel, F. C., Rothfuss, O. C., Kahle, P. J., et al. (2010). PINK1/Parkin-mediated mitophagy is dependenton VDAC1 and p62/SQSTM1. *Nat. Cell. Biol.* 12, 119–131. doi: 10.1038/ncb2012

Hall, A. M., and Schuh, C. D. (2016). Mitochondria as therapeutic targets in acute kidney injury. *Curr. Opin. Nephrol. Hypertens.* 25, 355–362. doi: 10.1097/MNH.0000000000000228

Huang, L. L., Long, R. T., Jiang, G. P., Jiang, X., Sun, H., Guo, H., et al. (2018). Augmenter of liver regeneration promotes mitochondrial biogenesis in renal ischemia-reperfusion injury. *Apoptosis* 23, 695–706. doi: 10.1007/s10495-018-1487-2

Ishihara, M., Urushido, M., Hamada, K., Matsumoto, T., and Shimamura, Y. (2013). Sestrin-2 and BNIP3 regulate autophagy and mitophagy in renal tubular cells in acute kidney injury. *Am. J. Physiol. Renal. Physiol.* 305, F495–F509. doi: 10.1152/ajprenal.00642.2012

Jiang, M., Liu, K., Luo, J., and Dong, Z. (2010). Autophagy is a renoprotective mechanism during in vitro hypoxia and in vivo ischemiareperfusion injury. *Am. J. Pathol.* 176, 1181–1192. doi: 10.2353/ajpath.2010.090594

Jiang, X., Liao, X. H., Huang, L. L., Sun, H., Liu, Q., and Zhang, L. (2019). Overexpression of augmenter of liver regeneration (ALR) mitigates the effect of H2O2-induced endoplasmic reticulum stress in renal tubule epithelial Cells. *Apoptosis* 24, 278–289. doi: 10.1007/s10495-019-01517-z

Kezic, A., Spasojevic, I., Lezaic, V., and Bajcetic, M. (2016). Mitochondria-targeted antioxidants: future perspectives in kidney ischemia reperfusion injury. *Oxid. Med. Cell. Longev.* 2016:2950503. doi: 10.1155/2016/2950503

LaBrecque, D. R., and Pesch, L. A. (1975). Preparation and partial characterization of hepaticregenerative stimulator substance (SS) from rat liver. *J. Physiol.* 248, 273–284. doi: 10.1113/jphysiol.1975.sp010973

Lange, H., Lisowsky, T., Gerber, J., Mühlenhoff, U., Kispal, G., and Lill, R. (2001). An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins. *EMBO. Rep.* 2, 715–720. doi: 10.1093/embo-reports/kve161

Liao, X. H., Zhang, L., Liu, Q., Sun, H., Peng, C. M., and Guo, H. (2010). Augmenter of liver regeneration protects kidneys from ischaemia/reperfusion injury in rats. *Nephrol. Dial. Transplant.* 25, 2921–2929. doi: 10.1093/ndt/gfq151

Liao, X. H., Zhang, L., Tang, X. P., Liu, Q., and Sun, H. (2009). Expression of augmenter of liver regeneration in rats with gentamicin-induced acute renal failure and its protective effect on kidney. *Ren. Fail.* 31, 946–955. doi: 10.3109/08860220903216154

Lin, Q., Li, S., Jiang, N., Shao, X., Zhang, M., Jin, H., et al. (2019). PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. *Redox. Biol.* 26:101254. doi: 10.1016/j.redox.2019.101254

Linkermann, A., Chen, G., Dong, G., Kunzendorf, U., Krautwald, S., and Dong, Z. (2014). Regulated cell death in AKI. *J. Am. Soc. Nephrol.* 25, 2689–2701. doi: 10.1681/ASN.2014030262

Liu, L., Feng, D., Chen, G., Chen, M., Zheng, Q., Song, P., et al. (2012). Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat. Cell. Biol.* 14, 177–185. doi: 10.1038/ncb2422

Malek, M., and Nematbakhsh, M. (2015). Renal ischemia/reperfusion injury; from pathophysiology to treatment. *J. Renal. Inj. Prev.* 4, 20–27. doi: 10.12861/jrip.2015.06

Marino, G., Niso-Santano, M., Baehrecke, E. H., and Kroemer, G. (2014). Self-consumption: the interplay of autophagy and apoptosis. *Nat. Rev. Mol. Cell. Biol.* 15, 81–94. doi: 10.1038/nrm3735

Mehta, R. L., Burdmann, E. A., Cerdá, J., Feehally, J., Finkelstein, F., García-García, G., et al. (2016). Recognition and management of acute kidney injury in the internationalsociety of nephrology 0by25 global snapshot: a multinationalcross-sectional study. *Lancet* 387, 2017–2025. doi: 10.1016/S0140-6736(16)30240-9

Mizumura, K., Cloonan, S. M., Nakahira, K., Bhashyam, A. R., Cervo, M., Kitada, T., et al. (2014). Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *J. Clin. Invest.* 124, 3987–4003. doi: 10.1172/JCI74985

Mordas, A., and Tokatlidis, K. (2015). The MIA pathway: a key regulator of mitochondrial oxidative protein folding and biogenesis. *Acc. Chem. Res.* 48, 2191–2199. doi: 10.1021/acs.accounts.5b00150

Nalesnik, M. A., Gandhi, C. R., and Starzl, T. E. (2017). Augmenter of liver regeneration: a fundamental life protein. *Hepatology* 66, 266–270. doi: 10.1002/hep.29047

Narendra, D., Kane, L. A., Hauser, D. N., Fearnley, I. M., and Youle, R. J. (2010). p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 6, 1090–1106. doi: 10.4161/auto.6.8.13426

Parikh, S. M., Yang, Y., He, L., Tang, C., Zhan, M., and Dong, Z. (2015). Mitochondrial function and disturbances in the septic kidney. *Semin. Nephrol.* 35, 108–119. doi: 10.1016/j.semnephrol.2015.01.011

Polimeno, L., Pesetti, B., De Santis, F., Resta, L., Rossi, R., De Palma, A., et al. (2012). Decrease dexpression of the augmenter of liver regeneration results inincreased apoptosis and oxidative damage in human-derived glioma cells. *Cell. Death. Dis.* 3:e289. doi: 10.1038/cddis.2012.25

Tang, C., Han, H., Yan, M., Zhu, S., Liu, J., Liu, Z., et al. (2018). PINK1-PRKN/PARK2 pathway of mitophagy is activated to protect against renal ischemia-reperfusion injury. *Autophagy* 14, 880–897. doi: 10.1080/15548627.2017.1405880

Tatsuta, T., and Langer, T. (2008). Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO. J.* 27, 306–314. doi: 10.1038/sj.emboj.7601972

Tury, A., Mairet-Coello, G., Lisowsky, T., Griffond, B., and Fellmann, D. (2005). Expression of the sulfhydryl oxidase ALR (augmenter of liver regeneration) in adult rat brain. *Brain. Res.* 1048, 87–97. doi: 10.1016/j.brainres.2005.04.050

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304, 1158–1160. doi: 10.1126/science.1096284

Venkatachalam, M. A., Weinberg, J. M., Kriz, W., and Bidani, A. K. (2015). Failed tubule recovery, AKI-CKD transition, and kidney disease progression. *J. Am. Soc. Nephrol.* 26, 1765–1776. doi: 10.1681/ASN.2015010006

Wong, Y. C., and Holzbaur, E. L. (2014). Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proc. Natl. Acad. Sci. U.S.A.* 111, E4439–E4448. doi: 10.1073/pnas.1405752111

Xia, N., Yan, R. Y., Liu, Q., Liao, X. H., Sun, H., Guo, H., et al. (2015). Augmenter of liver regeneration plays a protective role against hydrogen peroxide-induced oxidative stress in renal proximal tubule cells. *Apoptosis* 20, 423–432. doi: 10.1007/s10495-015-1096-2

Yang, X., Yan, X., Yang, D., Zhou, J., Song, J., and Yang, D. (2018). Rapamycin attenuates mitochondrial injury and renal tubular cell apoptosis in experimental contrast-induced acute kidney injury in rats. *Biosci. Rep.* 38:BSR20180876. doi: 10.1042/BSR20180876

Yu, J.-W., and Lee, M.-S. (2016). Mitochondria and the NLRP3 inflammasome: physiological and pathological relevance. *Arch. Pharm. Res.* 39, 1503–1518. doi: 10.1007/s12272-016-0827-4

Zhang, W., Siraj, S., Zhang, R., and Chen, Q. (2017). Mitophagy receptor FUNDC1 regulates mitochondrial homeostasis and protects the heart from I/R injury. *Autophagy* 13, 1080–1081. doi: 10.1080/15548627.2017.1300224

Zhang, Z. S., Yang, D. Y., Fu, Y. B., Zhang, L., Zhao, Q. P., and Li, G. (2015). Knockdown of CkrL by shRNA deteriorates hypoxia/reoxygenation-induced H9C2 cardiomyocyte apoptosis and survival inhibition Via Bax and downregulation of P-Erk1/2. *Cell. Biochem. Funct.* 33, 80–88. doi: 10.1002/cbf.3093

Zhao, C., Chen, Z., Xu, X., An, X., Duan, S., Huang, Z., et al. (2017). Pink1/Parkin-mediated mitophagy play a protective role in cisplatin induced renal tubular epithelial cells injury. *Exp. Cell. Res.* 350, 390–397. doi: 10.1016/j.yexcr.2016.12.015

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 07 July 2020
doi: 10.3389/fphys.2020.00732

The Vicious Cycle of Renal Lipotoxicity and Mitochondrial Dysfunction

*Mengyuan Ge[1,2], Flavia Fontanesi[3], Sandra Merscher[1,2] and Alessia Fornoni[1,2]**

[1]Katz Family Division of Nephrology and Hypertension, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL, United States, [2]Peggy and Harold Katz Family Drug Discovery Center, University of Miami Miller School of Medicine, Miami, FL, United States, [3]Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, United States

The kidney is one of the most energy-demanding organs that require abundant and healthy mitochondria to maintain proper function. Increasing evidence suggests a strong association between mitochondrial dysfunction and chronic kidney diseases (CKDs). Lipids are not only important sources of energy but also essential components of mitochondrial membrane structures. Dysregulation of mitochondrial oxidative metabolism and increased reactive oxygen species (ROS) production lead to compromised mitochondrial lipid utilization, resulting in lipid accumulation and renal lipotoxicity. However, lipotoxicity can be either the cause or the consequence of mitochondrial dysfunction. Imbalanced lipid metabolism, in turn, can hamper mitochondrial dynamics, contributing to the alteration of mitochondrial lipids and reduction in mitochondrial function. In this review, we summarize the interplay between renal lipotoxicity and mitochondrial dysfunction, with a focus on glomerular diseases.

Keywords: kidney, glomerular diseases, lipotoxicity, mitochondrial dysfunction, oxidative stress

OPEN ACCESS

Edited by:
Egor Plotnikov, Lomonosov Moscow State University, Russia

Reviewed by:
Mark Cooper, Monash University, Australia
Ilse Sofia Daehn, Icahn School of Medicine at Mount Sinai, United States

****Correspondence:***
Alessia Fornoni
afornoni@med.miami.edu

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *07 May 2020*
Accepted: *08 June 2020*
Published: *07 July 2020*

Citation:
Ge M, Fontanesi F, Merscher S and Fornoni A (2020) The Vicious Cycle of Renal Lipotoxicity and Mitochondrial Dysfunction. Front. Physiol. 11:732. doi: 10.3389/fphys.2020.00732

INTRODUCTION

Measurement of resting metabolic rates shows that the kidney and heart have the highest resting energy expenditure among major organs and tissues in adults (Wang et al., 2010). Mitochondria provide energy to support kidney functions such as removing waste and balancing fluid. The energy currency ATP is mostly generated by oxidative phosphorylation (OXPHOS) coupled to electron transport through the mitochondrial respiratory chain (MRC). Increasing evidence indicates that mitochondrial damage and dysfunction are major contributors to the pathogenesis of chronic kidney disease (CKD) (Che et al., 2014). Lipids serve as a source and reservoir of energy supply. However, lipid surplus can hamper mitochondrial dynamics, contributing to increased reactive oxygen species (ROS) production and mitochondrial dysfunction. Conversely, impaired mitochondrial function can disturb the balance between lipid availability and lipid use, further increasing lipid accumulation (Schrauwen-Hinderling et al., 2016). Excess renal lipids have been reported in CKD of different origin, suggesting the existence of renal lipotoxicity (Gai et al., 2019). Although it has been suggested that both lipid dysmetabolism and mitochondrial dysfunction contribute to CKD, it remains to be established whether renal lipotoxicity is the cause or the consequence of mitochondrial dysfunction in the pathogenesis of CKD.

While tubular cells are mainly responsible for fluid and waste balance and account for most of the energy metabolism in the kidney, mitochondrial dysfunction and lipotoxicity were recently also described in diseases of glomerular origin. This is of particular importance as, different from tubular cells, key glomerular cells forming the kidney filtration barrier such as podocytes are terminally differentiated and, once injured, are unlikely to be replaced, thus causing proteinuria and disease progression (Kriz et al., 1998). Unlike tubular epithelial cells which preferentially use fatty acid (FA) β-oxidation as the main energy source (Kang et al., 2015), podocytes, endothelial cells, and mesangial cells in glomeruli highly rely on glucose as the substrate for energy production, while FA is used as an alternative substrate (Abe et al., 2010; Fink et al., 2012; Czajka and Malik, 2016). In the diabetic kidney, glucose oxidation in glomerular cells is disrupted and could account for the switch to FA oxidation (Forbes and Thorburn, 2018). In support, Imasawa et al. reported an oxidative shift during podocyte differentiation, where the expression of most glycolytic enzymes is decreased while the expression of proteins that are involved in FA β-oxidation is up-regulated (Imasawa et al., 2017). Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α expression can also induce a shift from their baseline glucose preference toward FA usage in podocytes (Li et al., 2017). Glomerular diseases, either primary or secondary to systemic diseases, represent an important cause of CKD (D'Agati et al., 2011). While we previously discussed how lipid accumulation contributes to mitochondrial dysfunction (Ducasa et al., 2019a), the current review focusses on and highlights the "two-way" interaction between lipids and mitochondria. More precisely, we summarize the findings observed in the setting of glomerular diseases with the goal to link lipotoxicity and mitochondrial dysfunction to irreversible podocyte injury and glomerular diseases.

MITOCHONDRIAL FUNCTION AND BIOGENESIS

Mitochondria fulfill several pivotal roles in cellular metabolism. In aerobic organisms, mitochondria are mostly responsible for the conversion of nutrient-derived energy in the form of ATP molecules through the mitochondrion-housed pathways of the Krebs cycle, FA β-oxidation, and OXPHOS (Lenaz and Genova, 2010). The complete oxidation of sugars and fats to carbon dioxide is achieved in the Krebs cycle and is paired with the concomitant conservation of free energy in the form of the reducing equivalents, nicotinamide-adenine dinucleotide (NADH), and flavin-adenine dinucleotide ($FADH_2$). The metabolic intermediate oxidized in the Krebs cycle, acetyl-coenzyme A (acetyl-CoA), derives from pyruvate generated by glycolysis in the cytosol and from the breakdown of FA. FA β-oxidation occurs primarily in mitochondria through a series of four enzymatic reactions that constitute the predominant energy-producing pathway in the kidney (Bhargava and Schnellmann, 2017). In mitochondria, both the Krebs cycle and FA β-oxidation generate NADH and $FADH_2$, which in turn transfer electrons to the MRC. Here, energy is further converted into an electrochemical gradient across the mitochondrial inner membrane. This conversion is achieved by the coupling of electron transfer through the MRC components to the final electron acceptor, molecular oxygen, with proton translocation from the mitochondrial matrix to the intermembrane space. Lastly, the protein gradient is used as the source of energy to drive ATP synthesis. The MRC is formed by four multimeric complexes (CI–CIV) embedded in the mitochondrial inner membrane and two mobile electron carriers, coenzyme Q (CoQ) and cytochrome *c*. Together, the MRC and ATP synthase (or CV) constitute the mitochondrial OXPHOS system (Lenaz and Genova, 2010).

ROS are byproducts of mitochondrial respiration, generated by electron "leakage" to oxygen, mainly at the level of CI and CIII of the MRC, to form superoxide anion (Stowe and Camara, 2009). *In vivo*, superoxide radicals have a short half-life and are rapidly converted into hydrogen peroxide by the action of superoxide dismutases, SOD1 and SOD2, located in the mitochondrial intermembrane space and matrix, respectively. While at low levels, ROS fulfill physiological functions as signaling molecules, mitochondrial dysfunction is commonly associated with a cellular energy deficit and increased ROS production, leading to the oxidative damage of cellular structures (Vafai and Mootha, 2012). Free FA, when in excess, can act as mitochondrial respiration uncouplers and MRC CI inhibitors and can also cause oxidative stress. To avoid lipotoxicity, FA levels in mitochondria are tightly controlled. FAs are stored in cytosolic lipid droplets (LDs), which exhibit close physical and metabolic interactions with mitochondria (Aon et al., 2014).

Furthermore, mitochondrial dysfunction is also frequently characterized by structural and morphological alterations. Mitochondria form a complex and highly dynamic network inside cells, which is continuously shaped by the processes of mitochondrial fusion and fission (Lackner, 2014). Mitochondrial hyperbranching, fragmentation, and loss of the mitochondrial inner membrane invaginations, known as cristae, have been observed in pathological conditions associated with mitochondrial respiratory defects and stress (Vincent et al., 2016). In addition to their role in cellular energy production, mitochondria are a hub for anabolic reactions, since the Krebs cycle provides intermediates for a wide range of biosynthetic pathways. These include precursors for amino acids, nucleobases, the antioxidant molecule glutathione, and metal prosthetic group biosynthesis. Notably, the Krebs cycle intermediate citrate is exported into the cytosol, where it is used as a substrate for FA and cholesterol synthesis (Owen et al., 2002). Among lipids, the synthesis of cardiolipin (CL) takes place in mitochondria. CL is the signature lipid of the mitochondrial inner membrane, which has an essential structural role in cristae formation and is required for optimal MRC assembly and function (Claypool and Koehler, 2012).

Mitochondria are organelles of endosymbiotic origin and still retain their genome, the mitochondrial DNA (mtDNA), and gene expression machinery, the mitochondrial ribosomes. In humans, the mtDNA encodes for a complete set of tRNAs, the ribosomal RNAs, and a handful of proteins, all catalytic subunits of the OXPHOS system. It has been estimated that the mitochondrial proteome could comprise about 1,500 proteins (Vafai and Mootha, 2012). With only 13 polypeptides encoded

by the mtDNA, most of these proteins, including the remaining subunits of the OXPHOS system, are encoded by nuclear genes, synthesized in the cytosol, and imported into mitochondria. Mutations in both nuclear and mitochondrial genes are causes of major energetic deficits associated with severe hereditary diseases known as mitochondrial disorders (Turnbull and Rustin, 2016). While defects in nuclear genes follow Mendelian inheritance, mutations in the mtDNA are maternally transmitted. Since each cell contains hundreds to thousands of mtDNA molecules, wildtype and mutant genomes can co-exist, a condition known as heteroplasmy. Clinical manifestation appears only when the fraction of mutated molecules reaches a certain threshold, which can be tissue-specific (Turnbull and Rustin, 2016). Primary mitochondrial disorders are multisystemic diseases, although high energy demanding organs, such as heart and brain, are frequently the most affected. Kidneys also require large amounts of energy, mainly for tubular reabsorption (Hansell et al., 2013). In several instances, renal manifestations have been reported in patients affected by mitochondrial disorders (Finsterer and Scorza, 2017). In addition to primary mitochondrial diseases, mitochondrial dysfunction has been shown to be involved in the etiology and progression of numerous human pathologies, including kidney diseases and, in particular glomerular diseases, which are the focus of this review.

MAJOR GLOMERULAR DISEASES DUE TO MITOCHONDRIAL CYTOPATHIES

Genetic defects in mtDNA or nuclear DNA (nDNA) that encode mitochondrial proteins can result in mitochondrial cytopathies. Though most mitochondrial disorders with a renal phenotype are characterized by tubular dysfunction, mtDNA 3243A > G mutation and CoQ10 biosynthesis defects (due to nDNA mutation) are associated with two mitochondrial cytopathies primarily causing glomerular diseases, most commonly focal segmental glomerulosclerosis (FSGS).

Mitochondrial DNA Mutations Associated With Glomerular Diseases

Thirteen proteins are encoded by the mitochondrial genome, all of which are subunits of the MRC (Anderson et al., 1981; Di Donato, 2009). Mutations in mtDNA cause defects in the mitochondrial OXPHOS, resulting in energy deficiency and increased ROS. The most common mtDNA point mutation 3243A > G in the $tRNA^{LEU}$ gene is associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. Representing one of the most frequent mitochondrial disorders, MELAS syndrome could be considered as an underlying cause of primary FSGS, as several studies reported glomerular manifestation, often presents as FSGS (Kurogouchi et al., 1998; Cheong et al., 1999; Hotta et al., 2001; Gucer et al., 2005; Rudnicki et al., 2016; Narumi et al., 2018). In patients with MELAS syndrome and renal symptoms, the renal disease initially manifests with proteinuria and typical changes of the glomerular basement membrane (GBM) (Kurogouchi et al., 1998; Cheong et al., 1999; Narumi et al., 2018). Biopsy samples from these patients show abnormal mitochondria in podocytes, as well as foot process effacement (Gucer et al., 2005; Narumi et al., 2018). Patients with other point mutations (m.A4269G, m.A5728G, and m.A5843G) that affect mitochondrial tRNAs present with various severe clinical phenotypes, from fatal cardiomyopathy to multi-organ failure. These mutations are not associated with MELAS but were also reported to be associated with an FSGS phenotype (Taniike et al., 1992; Scaglia et al., 2003; Meulemans et al., 2006). Interestingly, deafness was also described in individuals with MELAS syndrome, which resembles the symptom of another rare genetic disease characterized by mutations of another important constituent of the GBM, i.e., type IV collagen (Alport syndrome). Besides, FSGS has been reported in a case of Kearns-Sayre syndrome due to mtDNA deletion (Becher et al., 1999). It is worth noting that mtDNA mutations associated with glomerular diseases affect overall mitochondrial gene expression and, in consequence, cause combined MRC enzymatic deficiencies, commonly associated with very severe outcomes.

Mutations in nDNA That Encodes Mitochondrial Proteins Associated With Glomerular Diseases

Besides the 13 proteins encoded by the mitochondrial genome, there are over 1,158 other nuclear-encoded mitochondrial proteins (Calvo et al., 2016). The enzymes of the CoQ10 biosynthetic pathway are encoded by nuclear *CoQ* genes. Located mainly in the mitochondrial inner membrane, CoQ10 is a carrier that transfers electrons from complex I and II to complex III of the MRC (Ernster and Dallner, 1995). At least 15 genes are required in CoQ10 biosynthesis (Desbats et al., 2015). Mutations in these genes may cause CoQ10 deficiency, some of which were reported to be associated with glomerular involvement. In humans, prenyl (decaprenyl) diphosphate synthase subunit 1 (PDSS1) and PDSS2 are responsible for the synthesis of the prenyl side chain of CoQ10 (Saiki et al., 2005). Deficiency in CoQ10 content was reported in a patient carrying compound heterozygous mutations in PDSS2. The patient was diagnosed with Leigh's syndrome with nephropathy (Lopez et al., 2006). Dietary supplementation with CoQ10 was shown to rescue proteinuria and interstitial nephritis in Pdss2kd/kd mice (Saiki et al., 2008) and diabetic nephropathy in db/db mice (Sourris et al., 2012). Gasser et al. analyzed patients with FSGS and collapsing glomerulopathy for PDSS2 polymorphisms. Surprisingly, they found a reduction of CoQ10 content, irrespectively of the PDSS2 haplotype (Gasser et al., 2013). Vasta et al. also reported that one patient carrying a PDSS1 mutation presented with nephrotic syndrome (Vasta et al., 2012). Pathogenic CoQ2 variants are another cause of primary CoQ10 deficiency. Patients with CoQ2 mutations have been reported to exhibit nephropathy that can be treated by CoQ10 supplementation (Starr et al., 2018). Moreover, loss-of-function mutations in CoQ6 have been described as a cause of glomerular damage, and patients manifested with FSGS in renal biopsy (Heeringa et al., 2011). It is important to note that genetic testing in patients with steroid-resistant nephrotic syndrome identified mutations in several genes involved in mitochondrial function and CoQ10 biosynthesis, including AarF

domain-containing kinase 4 (ADCK4), CoQ6, CoQ2, and PDSS2 (Lovric et al., 2016). Patients who carry mutations in the ADCK4 gene may present with CoQ10 deficiency and mitochondrial nephropathy manifesting as FSGS (Ashraf et al., 2013; Yang et al., 2018; Widmeier et al., 2020). ADCK4 interacts with members of the CoQ10 biosynthesis pathway and is involved in CoQ biosynthesis, although its function remains to be characterized.

MITOCHONDRIAL DYSFUNCTION RESULTS IN RENAL LIPOTOXICITY

Mitochondrial dysfunction can lead to lipid metabolism disorders in several cell types. Vankoningsloo et al. studied preadipocytes incubated with inhibitors of mitochondrial respiration (antimycin A) and found that mitochondrial dysfunction induces triglyceride accumulation. This process is mediated by reduced PPARγ activity leading to decreased expression of muscle isoform of carnitine palmitoyltransferase I (CPT1) and FA β-oxidation. Direct conversion of glucose into triglycerides and activation of carbohydrate-responsive element-binding protein (ChREBP), a lipogenic transcription factor, were also observed (Vankoningsloo et al., 2005). Boren and Brindle observed that the induction of apoptosis leads to mitochondrial ROS generation in murine lymphoma cells and mouse embryo fibroblasts, which inhibits mitochondrial FA β-oxidation but increases acyl-CoA synthetase activity for FA synthesis. Thereby, the switch from FA β-oxidation into lipid synthesis results in LD accumulation in the cytoplasm (Boren and Brindle, 2012).

Dysregulated Mitochondrial Lipid Utilization in Kidney Diseases

Mitochondrial FA β-oxidation is the major energy pathway of the kidney. Mitochondrial dysfunction leads to compromised lipid utilization, eventually resulting in lipid accumulation, which is toxic to cells in the kidney. Herman-Edelstein et al. measured genes and enzymes involved in the FA β-oxidation pathway in kidneys of patients with diabetic nephropathy compared to healthy kidneys. The genetic analysis revealed the downregulation of some key transcriptional regulators of FA β-oxidation, such as PPARα, PPARγ, CPT1, and Aconitase 2 (ACO2) (Herman-Edelstein et al., 2014). Interestingly, upregulation of low-density lipoprotein receptor (LDLR) and cluster of differentiation 36 (CD36), downregulation of liver X receptor α (LXRα), ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1), and apolipoprotein E (APOE) were also detected, indicating alterations in cholesterol metabolism (**Figure 1**; Herman-Edelstein et al., 2014). In addition to diabetic nephropathy phenotypes including podocyte process effacement, thickening of the GBM, and mesangial expansion, electron microscopy analysis also showed extensive accumulation of LDs in podocytes, tubular epithelial cells, mesangial cells, and fenestrated endothelial cells (Herman-Edelstein et al., 2014). Consistently, CD36, PPARα, PPARγ, and LDLR were found to be genes with the highest connectivity to diabetic nephropathy progression in a study comparing human-mouse cross-species glomerular transcriptional networks (Hodgin et al., 2013).

A renal-protective effect of PPARs was also reported. The study by Calkin et al. suggests that the administration of PPARα, PPARγ, and PPARα/γ agonists in experimental mouse models of diabetes has renoprotective effects (Calkin et al., 2006). Doxorubicin is an antitumor drug that induces renal injury, leading to proteinuria. Doxorubicin-induced PPARα knockout mice showed more severe podocyte foot process effacement compared with Doxorubicin-induced wildtype mice but the treatment with PPARα agonist fenofibrate reduced proteinuria and ameliorated podocyte foot process effacement (Zhou et al., 2011). Fenofibrate has also been shown to prevent high-fat diet (HFD)-induced glomerular injury in an experimental model of diabetic nephropathy (Tanaka et al., 2011). Fenofibrate treatment reduces glomerular lipid (mainly triglycerides) accumulation, attenuates oxidative stress in kidneys of HFD-fed mice, and enhances the expression of lipolytic enzymes, including CPT1. Fenofibrate also attenuates tubulointerstitial injury by enhancing renal lipolysis (Tanaka et al., 2011). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, a trend to renoprotection was observed in fenofibrate treated patients with diabetic nephropathy (Keech et al., 2005). PPARγ is also involved in renal lipid metabolism. In fact, PPARγ expression is reduced in glomeruli of diabetic mice, while activation of PPARγ inhibits the development of diabetic glomerular lesion (Zheng et al., 2002). Moreover, another study demonstrated that both PPARα and PPARγ agonists increase LXRα and ABCA1 gene expression and enhance apolipoprotein A1 (APOA1)-mediated cholesterol efflux from human mesangial cells (Ruan et al., 2003). However, PPARγ2 KO mice generated in an ob/ob background exhibit lipid accumulation at both the glomerular and tubular levels, accompanied by glomerular damage, in addition to the original metabolic syndrome phenotype (Martinez-Garcia et al., 2012). Located in the mitochondrial inner membrane, uncoupling proteins (UCPs) are mitochondrial transporters that control the level of coupled respiration (Rousset et al., 2004). Ke et al. reported that UCP2 expression is increased in human biopsy samples and mice kidney tissues with tubulointerstitial fibrosis but UCP2 deficient mice display reduced lipid accumulation and attenuated hypoxia in kidney tissue. Compared with wildtype mice, the expression of PPARα and CPT1α, which are the major regulators of lipid metabolism, is restored in UCP2 deficient mice (Ke et al., 2020). Both CPT1 and ACO2 are the rate-limiting enzymes of FA β-oxidation. Idrovo et al. demonstrated that stimulating CPT1 activity with a synthetic compound (C75) not only restores ATP generation but also improves renal function using a rat model of ischemia/reperfusion injury (Idrovo et al., 2012). In addition, mRNA levels of ACO1 and ACO2 are decreased in the tubulointerstitial and the glomerular compartment of non-diabetic CKD patients when compared to healthy controls (Hallan et al., 2017).

Oxidative Stress Causes Renal Lipid Accumulation

Free FAs are sources of energy to synthesize ATP by mitochondrial FA β-oxidation. Palmitic acid (PA) is the predominant circulating saturated FA, which plays an important role in podocyte injury

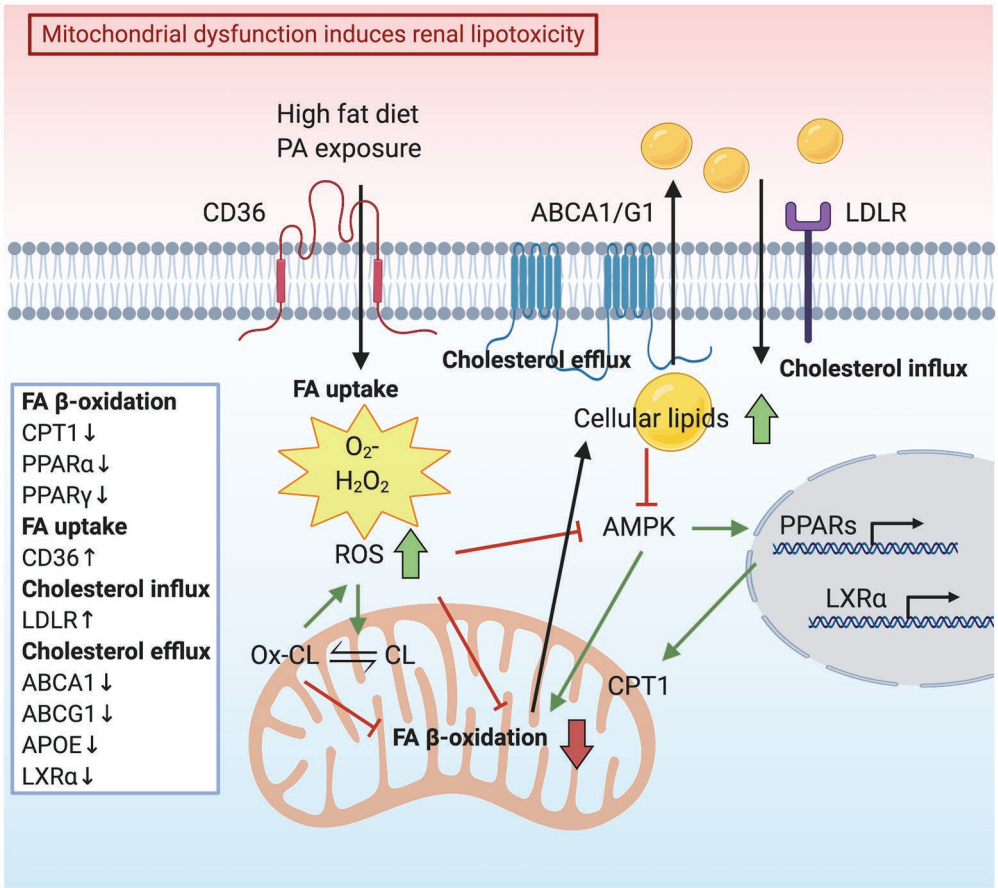


FIGURE 1 | Schematic representation indicating how mitochondrial dysfunction induces renal lipotoxicity. High-fat diet or palmitic acid (PA) exposure increases mitochondrial reactive oxygen species (ROS) production. Consequently, elevated oxidative stress reduces mitochondrial fatty acid (FA) oxidation, resulting in lipid accumulation in the cytoplasm. Lipid accumulation is also caused by deceased cholesterol efflux mediated by ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) and elevated cholesterol influx *via* low-density lipoprotein receptor (LDLR). AMP-activated protein kinase (AMPK) suppression due to a lipid surplus inhibits mitochondrial FA oxidation and lipid utilization, accompanied by downregulation of transcription factors, liver X receptor (LXR) α, peroxisome proliferator-activated receptor (PPAR) α, and PPARγ. ROS generation contributes to cardiolipin (CL) peroxidation and loss of mitochondria cristae membranes, thus impairing the capacity for energy production.

through lipotoxicity initiated by mitochondrial superoxide overproduction (Lee et al., 2017). Podocytes are vulnerable to PA-induced oxidative damage due to impaired peroxidase activity. Podocytes incubated with PA show increased FA uptake, mitochondrial superoxide, and hydrogen peroxide (H_2O_2) generation, as well as elevated AMP-activated protein kinase (AMPK) α phosphorylation (**Figure 1**; Lee et al., 2017). Furthermore, Liu et al. demonstrated that PA induces intracellular lipid accumulation and LD formation. It also induces podocyte apoptosis through activation of the mitochondrial pathway and triggers the release of cytochrome *c* from the mitochondria to the cytosol (Liu et al., 2018). In mouse podocytes, PA induces apoptosis by increasing cytosolic Ca^{2+} concentration, resulting from Ca^{2+} accumulation in the mitochondria *via* the mitochondrial Ca^{2+} uniporter (Yuan et al., 2017). The depletion of endoplasmic reticulum (ER) Ca^{2+} is also due to oxidative stress caused by PA (Xu et al., 2015). Under diabetic conditions, podocytes are susceptible to PA-induced oxidative damage due to an inadequate activity of peroxidase, the enzyme that catalyzes the conversion of the intracellular H_2O_2 to water in response to excess ROS generation (Lee and Lee, 2018). On the contrary, the nontoxic mono-unsaturated FA oleic acid (OA) inhibits the PA-induced ROS formation in podocytes (Lee et al., 2017; Lee and Lee, 2018). OA attenuates ROS generation and protects the mitochondria from PA-induced oxidative stress by the restoration of AMPK activity (Palomer et al., 2018).

Oxidative stress in the form of lipid peroxidation can be caused by the generation of lipid peroxyl radicals in podocytes. Excessive accumulation of lipid peroxyl radicals contributes to podocyte injury in CKD (Kruger et al., 2018). It was demonstrated that ROS generation contributes to glomerular and tubular injury through lipid peroxidation of cell and organelle membranes. ROS-induced lipid peroxidation disrupts the structural integrity of the lipid bilayer and impairs the capacity for energy production (Baud and Ardaillou, 1993). SS-31 is a tetrapeptide that protects mitochondria cristae structure and matrix density in all kidney cells. SS-31 targets CL, the phospholipid almost exclusively present in the inner mitochondrial membrane (Szeto and Birk, 2014).

CL peroxidation alters mitochondrial cristae formation (Szeto, 2014), thus disrupting the functional and structural integrity of respiratory supercomplexes (Pfeiffer et al., 2003). Moreover, during apoptosis, CL binds to cytochrome *c* to form peroxidase complexes capable of catalyzing CL peroxidation and promoting cytochrome *c* release from mitochondria (Kagan et al., 2005). SS-31 selectively binds to CL, preventing CL from converting cytochrome *c* into a peroxidase while maintaining its function as an electron carrier (Szeto, 2014). The study of Szeto et al. described changes in mitochondrial structure in glomerular endothelial cells, podocytes, and proximal tubular epithelial cells after HFD induction. HFD increases mitochondrial ROS generation, causing CL peroxidation and loss of mitochondria cristae membranes. Increased ROS limits mitochondrial FA β-oxidation, which in turn causes cellular lipid accumulation and inhibits AMPK activity (**Figure 1**; Szeto et al., 2016). By preserving normal mitochondrial cristae structure, SS-31 treatment was able to restore AMPK activity and prevent intracellular lipid accumulation, ER stress, and glomerular inflammation (Szeto et al., 2016). AMPK is a cellular energy sensor that regulates glucose, lipid, and protein metabolism. During energy shortage, AMPK is activated and promotes ATP production (Hardie et al., 2012). On the contrary, AMPK is down regulated under conditions of energetic oversupply (Picard et al., 2012). Mice fed on a HFD show increased glomerular area and matrix accumulation. A significant rise of cholesteryl esters and phospholipids in the kidneys was also observed, in association with reduced AMPK activity. Reduced AMPK activity was also described in kidneys from both diabetic mice and in patients with diabetes (Dugan et al., 2013). Conversely, the activation of AMPK reverses the accumulation of cholesteryl esters and phospholipids in kidneys of mice fed a HFD (Decleves et al., 2011, 2014). Improving mitochondrial biogenesis and function by AMPK activation was found to be a therapeutic approach in a rat model of diabetic nephropathy (Zhou et al., 2019). The administration of AMPK activators increased the expression of PGC-1α, which induces a ROS scavenging mechanism, including the expression of SOD2 in the presence of ROS, protecting cells against ROS generation and damage (Spiegelman, 2007; Zhou et al., 2019). Similarly, AMPK activation in diabetic mice restores mitochondrial content and function while improving DKD progression (**Figure 1**; Dugan et al., 2013).

INHERITED LIPID DISORDERS ARE ASSOCIATED WITH RENAL INJURY

Defects in genes relevant to lipid metabolism result in inherited diseases associated with renal lipotoxicity. Tangier disease is a rare disorder characterized by reduced levels of high-density lipoprotein (HDL) in the blood. Patients with Tangier disease have mutations in the ABCA1 gene and present with mild proteinuria and foamy podocytes in kidney biopsy (Ferrans and Fredrickson, 1975; Schaefer et al., 2010). Similarly, foamy podocytes, vacuolated tubular epithelial cells, and interstitial foam cells were described in a case report of a patient with Niemann-Pick disease, an inherited condition with an accumulation of sphingomyelin in various organs, including kidneys (Grafft et al., 2009). Caused by the accumulation of cholesterol in blood and tissue, familial lecithin-cholesterol acyltransferase (LCAT) deficiency is another genetic disorder, where some patients present with kidney injury. Renal biopsies of patients with LCAT deficiency identified LDs in mesangial and endothelial cells (Ossoli et al., 2015). Borysiewicz et al. described that patients with LCAT deficiency show disturbance of lipid metabolism and accumulation of lipids in the kidneys, eventually leading to end-stage kidney disease (Borysiewicz et al., 1982). In addition, transgenic mice with LCAT deficiency develop proteinuria and glomerulosclerosis characterized by the accumulation of free cholesterol and polar lipids in glomeruli (Lambert et al., 2001). Disturbed lipid metabolism was also reported in rare cases of lipoprotein glomerulopathy. Patients with an APOE gene mutation are characterized by proteinuria and hyperlipidemia. It has been suggested that the mutation of APOE increases the affinity of lipoproteins for glomerular capillary walls and promotes the formation of lipoprotein aggregates (Sam et al., 2006; Tsimihodimos and Elisaf, 2011). The evidence that genetic impairment of genes regulating cholesterol efflux leads to glomerular disease strongly suggests a causative link between altered renal lipid metabolism and kidney disease. This is very different from genetic disorders of hyperlipidemia, such as familial hypercholesterolemia (FH), where the high level of circulating lipids does not necessarily translate into an increased accumulation of renal lipids and does not always cause a renal phenotype. While this remains controversial, a recent association between FH and CKD was reported (Emanuelsson et al., 2018), and a patient with homozygous FH was found to develop FSGS (Elmaci et al., 2007). Similarly, the accumulation of lipids was found in the renal parenchyma of patients affected by FH (Buja et al., 1979). Although some of these studies suggest a link between systemic hyperlipidemia and CKD, most of the studies have demonstrated that statins may partially reduce proteinuria but this does not result in the prevention of CKD progression (Agarwal, 2006).

GLOMERULAR LIPID ACCUMULATION RESULTS IN MITOCHONDRIAL DYSFUNCTION

Podocytes rely on a constant supply of lipids and proteins to form foot processes (Simons et al., 1999; Fornoni et al., 2014). However, lipid overload causes lipotoxicity. Lipid accumulation and increased inflammation accelerate lipotoxicity induced glomerular disease (Martinez-Garcia et al., 2015). Among glomerular cells, podocytes are terminally differentiated cells that are most susceptible to damage caused by lipid overload. The podocyte slit diaphragm is assembled as a lipid-raft like structure, enriched with lipids including sphingolipids and cholesterol. Plasma membrane and intracellular lipids can affect podocyte function (Fornoni et al., 2014). Localized to lipid raft domain, sphingomyelinase-like phosphodiesterase 3b (SMPDL3b) is a sphingolipid-related enzyme that modifies the plasma lipid composition and modulates intracellular inflammatory pathways in podocytes (Heinz et al., 2015; Yoo et al., 2015). Moreover, cholesterol is required for the proper

function of the slit diaphragm (Simons et al., 2001), while excessive accumulation of cholesterol causes podocyte injury and proteinuria (Merscher-Gomez et al., 2013; Pedigo et al., 2016).

Hyperlipidemia and Hyperlipidemia-Induced Lipotoxicity

Foam cells are macrophages that ingest low-density lipoprotein (LDL) during hyperlipidemia. Their formation represents the hallmark of atherosclerosis (Yu et al., 2013). Interestingly, foam cells also appear in various glomerular diseases, including diabetic nephropathy, FSGS, nephrotic syndrome, and Alport syndrome (Nolasco et al., 1985; Stokes et al., 2006; Wu et al., 2009; Eom et al., 2015), indicating lipid-mediated toxicity. However, it is not established whether foam cells generated during the pathogenesis of kidney diseases are renal resident cells or infiltrating macrophages from the circulation (Eom et al., 2015). It is certain that glomerular cells can uptake LDL. In fact, the activity of lipoprotein receptors in cultured epithelial cells of the human glomerulus was characterized in the early 1990s. Grone et al. demonstrated that cultured glomerular epithelial cells show lipoprotein receptor activity, which mediates the uptake of apolipoprotein B (APOB)- and APOE-rich lipoproteins, contributing to lipid accumulation and oxidative stress (Grone et al., 1990). In addition, the accumulation of APOB and APOE in immune deposits could lead to GBM damage (**Figure 2**). Heymann nephritis is a rat model of membranous nephropathy characterized by the formation of subepithelial immune deposits in the GBM (Heymann et al., 1959). In Heymann nephritis, Kerjaschki et al. demonstrated that antibodies targeting megalin (an LDL-related protein) inhibit megalin-mediated binding and clearance of APOE and APOB, leading to the accumulation of apolipoproteins in immune deposits. The lipid environment is associated with lipid peroxidation, injury of the GBM, and proteinuria (Kerjaschki et al., 1997). Oxidized LDL has also

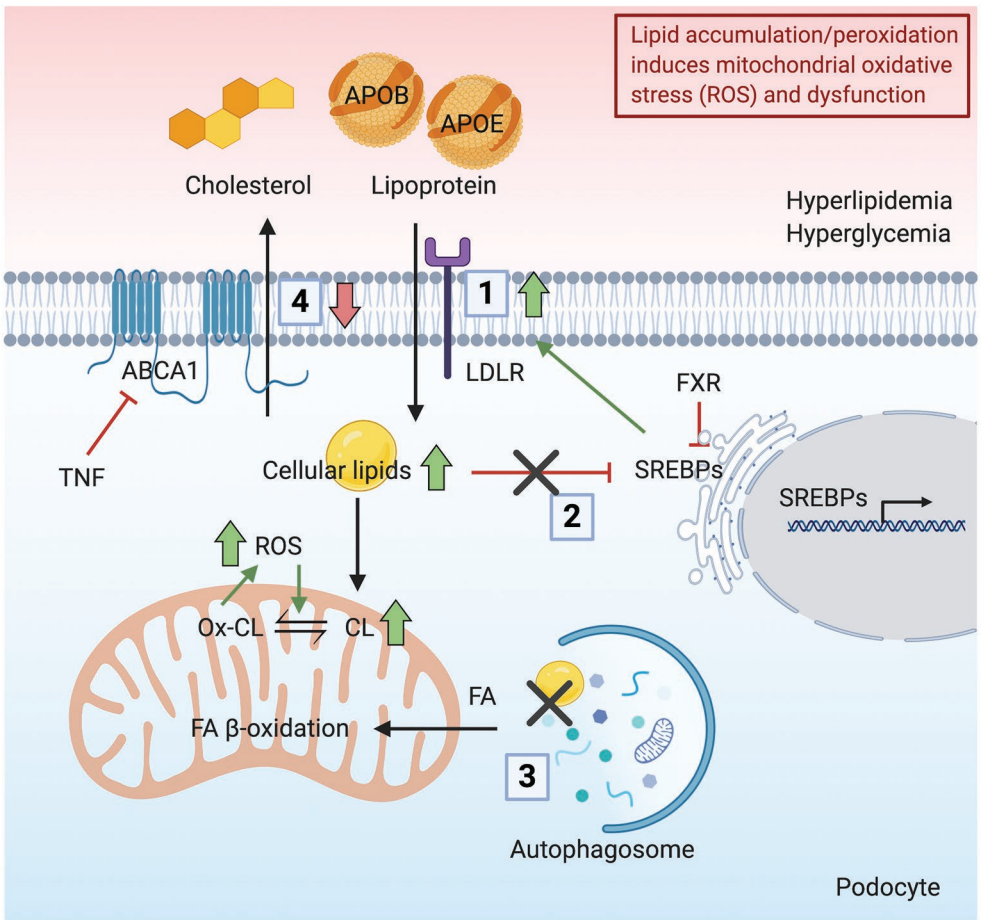


FIGURE 2 | Schematic representation indicating how lipid accumulation/peroxidation induces mitochondrial oxidative stress and dysfunction. Podocyte lipid accumulation and increased peroxidation induces ROS production and mitochondrial dysfunction. (1) In the presence of hyperlipidemia, increased uptake of apolipoprotein B (APOB)- and apolipoprotein E (APOE)-rich lipoproteins occurs in podocytes through the LDLR, resulting in a lipid-rich environment. (2) Hyperglycemia disrupts the feedback regulation of sterol regulatory element-binding proteins (SREBPs), causing further lipid accumulation. (3) Cellular lipids, mainly in the form of triglycerides, stored in lipid droplets (LDs) are hydrolyzed and delivered as FAs to mitochondria by autophagic clearance. The inhibition of autophagic clearance of lipids is associated with increased lipid endocytosis. (4) Downregulation of ABCA1 impairs reverse cholesterol transport, resulting in the accumulation of cholesterol and CL and peroxidation of CL. Together, the accumulation of lipids in podocytes induces ROS production and mitochondrial dysfunction.

been found to directly damage podocytes *via* the chemokine ligand 16 (CXCL16), as well as induce ROS production in podocytes (Bussolati et al., 2005; Gutwein et al., 2009a). CXCL16 is a major scavenger receptor for oxidized LDL in human podocytes. Both glomerular levels of CXCL16 and oxidized LDL are increased in patients with diabetic nephropathy and membranous nephropathy (Gutwein et al., 2009a,b).

Experimental evidence also supports a role of lipid-lowering agents in the protection of kidney diseases. For example, treatment of patients with lipid-lowering agents such as the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor atorvastatin was able to lower serum lipids and glomerular lesions but to a lesser extent than the blockage of the renal angiotensin system with quinapril. In fact, the renal protective effect of atorvastatin is associated with lipid-related and non-lipid-related effects (Blanco et al., 2005). Once more, however, the renal protective effects of statin treatment remain to be confirmed in patients affected by CKD. Interestingly, hypercholesterolemia associated with nephrotic syndrome can be ameliorated by inhibiting proprotein convertase subtilisin/kexin type 9 (PCSK9), a posttranscriptional regulator of the LDL receptor. PCSK9 targets LDLR for degradation and thereby reduces the clearance of LDL from the circulation (Shrestha et al., 2019). Inhibition of PCSK9 decreases the proportion of APOB-associated cholesterol (VLDL and LDL) and increases the proportion of HDL-associated cholesterol, producing a more protective lipoprotein profile (Haas et al., 2016). Currently, the monoclonal antibodies evolocumab and alirocumab are approved PCSK9 inhibitors for therapeutic use and are effective and safe in patients with mild to moderate CKD (Schmit et al., 2019). However, it would be important to study if PCSK9 inhibitors can also alleviate proteinuria and CKD progression in patients with advanced CKD, such as nephrotic syndrome. Studies in cultured vascular smooth muscle and endothelial cells suggest that inhibition of PCSK9 attenuates ROS production, while PCSK9 overexpression increases ROS generation in a concentration-dependent manner (Ding et al., 2015). The crosstalk between PCSK9 and mtDNA damage in vascular smooth muscle cells is thought to be partially mediated by mitochondria-derived ROS (Ding et al., 2016). Lastly, more studies are needed to assess the contribution of LDL apheresis to the treatment of proteinuric kidney disease, in order to gain a better understanding of whether the antiproteinuric effects are linked to LDL apheresis or related proteins.

Intrarenal Lipid Accumulation in Glomerular Diseases

Lipid distribution among plasma, tissues, and cellular organelles can be altered by inflammatory stress (Ruan et al., 2009). In addition to the uptake of plasma lipids into cells *via* lipoprotein receptors, other factors that mediate lipid accumulation in glomerular cells, thus contributing to disease development and progression, have been described in several kidney diseases. Among them, we have demonstrated that glomerular TNF is a major driver of lipid dysmetabolism in FSGS (Pedigo et al., 2016). Our group reported the accumulation of cholesterol in kidney cortices in mouse models of diabetes (Ducasa et al., 2019b), Alport syndrome, and FSGS (Pedigo et al., 2016; Mitrofanova et al., 2018). Impaired ABCA1 mediated reverse cholesterol transport in a mouse model of FSGS was rescued by genetic ABCA1 overexpression. Similarly, treatment with hydroxypropyl-β-cyclodextrin (HPβCD) was found to reduce cholesterol accumulation in kidney cortices and was found to protect from renal failure in mouse models of FSGS, Alport syndrome, and DKD (Pedigo et al., 2016; Mitrofanova et al., 2018). Additionally, lipid accumulation is associated with the downregulation of ABCA1 in podocytes treated with sera from patients diagnosed with type 1 diabetes or type 2 diabetes (Pedigo et al., 2016; Ducasa et al., 2019b). Moreover, podocyte-specific deletion of ABCA1 renders mice susceptible to DKD progression, while the induction of ABCA1 ameliorates podocyte injury (Ducasa et al., 2019b).

Apart from failing to remove lipids from cells, lipid oversupply is another major cause of renal dysfunction (**Figure 2**). Sterol regulatory element-binding proteins (SREBPs) activate genes involved in the synthesis and uptake of cholesterol, FAs, triglycerides and phospholipids, endocytosis of LDL, and glucose metabolism (Edwards et al., 2000; Horton et al., 2002). In DKD, increased expression of SREBPs is associated with the accumulation of triglycerides and cholesterol in the kidney, resulting in glomerulosclerosis and proteinuria (Sun et al., 2002). Similarly, C57BL/6J mice fed on a HFD also show renal accumulation of triglycerides and cholesterol, accompanied by glomerulosclerosis due to SREBPs pathway activation (Jiang et al., 2005). Moreover, LDLR plays a role in the feedback system that modulates plasma and intracellular cholesterol homeostasis. The expression of LDLR is tightly regulated by SREBPs and SREBP cleavage-activating protein (SCAP). In the presence of high glucose or inflammatory stress, the LDLR feedback regulation is disrupted, causing intracellular cholesterol accumulation and podocyte injury (Zhang et al., 2015a,b). Interestingly, the up-regulation of hepatic PCSK9 was found to be modulated by SREBP-2 and SREBP-1c, which could furthermore dysregulate LDL cholesterol (Jeong et al., 2008; Rong et al., 2017).

Lipid Burden and Mitochondrial Dysfunction

The turnover of lipids in podocytes regulates podocyte health. Serezani reported that the expression of phosphatase and tensin homolog (PTEN) inhibits phagocytosis, cell growth, and cytoskeletal remodeling (Serezani et al., 2012). The downregulation of PTEN is associated with increased lipid endocytosis in podocytes from patients with obesity-related glomerulopathy, in cultured mouse podocytes with PTEN knock-down and in mice with podocyte-specific knockout of PTEN, thus enhancing the uptake of lipids and causing podocyte injury and proteinuria through oxidative stress response (Shi et al., 2019). On the contrary, elevated autophagic sequestration of LDs was reported as an adaption to alcoholic liver disease (Eid et al., 2013). In the study of Sato et al., podocytes with a higher level of autophagy are more competent to remove proteins and lipids from cells, suggesting a tendency to better prognosis (Sato et al., 2006). Autophagy related 5 (ATG5) is a critical autophagy gene during nephrogenesis. Mutation of ATG5 in the kidney epithelium leads to FSGS in mice, accompanied by enhanced ROS generation,

ER stress, and mitochondrial dysfunction (Kawakami et al., 2015). Interestingly, autophagy regulates lipid metabolism by autophagic clearance of cellular lipids stored as triglycerides in LDs, which are then hydrolyzed and delivered as FAs for energy production in mitochondria (**Figure 2**; Singh et al., 2009).

Lipid surplus induces a vicious cycle where the accumulation of lipids induces ROS production and mitochondrial dysfunction. Conversely, dysfunctional mitochondria may decrease the lipid oxidative capacity, which additionally increases the lipid surplus (Schrauwen-Hinderling et al., 2016). In a rat model of hypertension and proteinuria, increased metabolic stress was indicated by increased oxidized lipids derived from lipid breakdown in glomeruli at the early stage, causing decreased ATP and NADH levels. The change of metabolic aspects in glomeruli from these mice was associated with the activation of phosphoprotein-dependent signaling such as mTOR and AMPK (Rinschen et al., 2019). Steinberg et al. demonstrated that AMPK activity is suppressed by TNFα signaling, resulting in decreased FA β-oxidation and excess lipids in skeletal muscle (Steinberg et al., 2006). Notably, we previously showed that serum TNFR1 and TNFR2 levels are increased in patients with DKD and FSGS, whereas TNF levels are only increased in patients with DKD. More importantly, we demonstrated that glomerular TNF expression rather than systemic TNF causes free cholesterol accumulation and podocyte injury (Pedigo et al., 2016). High lipid availability induced ROS production can furthermore cause lipid oxidation (Schrauwen-Hinderling et al., 2016). Fibroblasts from patients with Tangier disease, who have an inherited ABCA1 mutation, show an increased content of the mitochondrial-specific phospholipid CL, while patients have reduced HDL levels in the blood (Fobker et al., 2001). Our group demonstrated that ABCA1 deficiency in human podocytes leads to CL accumulation and mitochondrial dysfunction. SS-31 has been used to scavenge ROS and stabilize CL (Zhao et al., 2004). The administration of an Abca1 inducer or elamipretide (SS-31) reduces CL peroxidation and ameliorates podocyte injury, indicating that ABCA1 deficiency causes CL accumulation and peroxidation, eventually leading to mitochondrial dysfunction (**Figure 2**; Ducasa et al., 2019b).

Reduction of renal parenchymal lipids ameliorates mitochondrial dysfunction, thus mitigating kidney injury. FSGS with podocyte injury and depletion can be mediated by mitochondrial oxidative damage in adjacent endothelial cells *via* endothelin-1 (EDN1) signaling. Podocyte-derived EDN1 can act on endothelial cells, which mediates oxidative stress and endothelial dysfunction *via* EDN1 receptor type A (EDNRA) activation, thus promoting podocyte apoptosis. In support of this observation, mitochondrial-targeted ROS scavengers and endothelin antagonists inhibit the release of EDN1, thus reducing mitochondrial oxidative stress and endothelial cell dysfunction, thereby preventing glomerulosclerosis (Daehn et al., 2014). In DKD, glomerular endothelial mitochondrial dysfunction is also associated with increased glomerular EDNRA expression and increased circulating EDN1. EDNRA antagonist treatment prevents mitochondrial oxidative damage in endothelial cells and ameliorates diabetes-induced glomerular injury, suggesting a crosstalk between podocytes and glomerular endothelial cells (Qi et al., 2017). Interestingly, EDN1 promotes lipid accumulation during the transformation of macrophage foam cells through the downregulation of ABCG1 and impaired HDL-mediated cholesterol efflux (Lin et al., 2011). It is possible that the inhibition of EDN1 signaling could promote reduced renal lipotoxicity in addition to suppressing oxidative stress. The farnesoid X receptor (FXR) is a bile acid-activated nuclear receptor that regulates lipid metabolism by modulating renal SREBP-1 activity. Since the SREBPs play a critical role in renal lipid accumulation, the administration of FXR agonists ameliorates renal triglyceride accumulation and regulates renal lipid metabolism in HFD-fed mice by modulating FA synthesis and oxidation, improving proteinuria and kidney injury (Wang et al., 2009). In addition, administration of the dual agonist of FXR and G protein-coupled membrane receptor Takeda G protein-coupled receptor 5 (TGR5) to mice decreases proteinuria and prevents mitochondrial impairments. This dual agonist modulates renal lipid metabolism and prevents renal lipid accumulation in mouse models of nephropathy associated with diabetes and obesity. Dual agonist treatment prevents inflammation, oxidative stress, and ER stress, as well as induces mitochondrial biogenesis and metabolism pathways (Wang et al., 2017, 2018). Similarly, Gai et al. also reported that FXR activation reduces glomerulosclerosis and tubulointerstitial injury in mice fed on a HFD. FXR agonist treated mice showed improved renal function in association with reduced lipid accumulation and reduced ROS. Additionally, FXR activation attenuated the decrease in autophagosomes in obese mice, maintaining effective energy production (Gai et al., 2016).

CONCLUSION

Lipids are essential sources for mitochondrial energy production. However, abnormal lipid metabolism in many tissues or cell types has proven to be detrimental and to contribute to the pathogenesis and progression of several disorders. We first summarized glomerular diseases caused by inherited mitochondrial disparities, as well as by inherited lipid disorders, thus providing the genetic evidence in humans that both altered mitochondrial function and lipid metabolism may contribute to the pathogenesis of kidney diseases. We then elaborated on the independent impacts of lipotoxicity and mitochondrial dysfunction on kidney diseases, especially diseases characterized by glomerular injury. Finally, we reviewed the evidence that lipotoxicity can be the cause or the consequence of mitochondrial dysfunction. Lipids form the bilayer membrane structure of mitochondria. However, the accumulation of lipids induces excess ROS generation and increased mitochondrial oxidative stress. Meanwhile, accumulation and peroxidation of CL interrupt the normal structure of mitochondria and impair mitochondrial respiration and energy production (**Figure 1**). On the other hand, mitochondria utilize lipids as a substrate for FA β-oxidation in order to meet high energy demand. Increased ROS production, secondary to stress conditions, inhibits mitochondrial FA β-oxidation and lipid utilization, which in turn generates more

ROS (**Figure 2**). It is clear that ROS play a critical role in the interplay of mitochondrial dysfunction and glomerular lipotoxicity. Interweaved by ROS production, a vicious cycle is formed by down-regulated FA β-oxidation, accumulation of intracellular lipids and peroxidation of lipids (especially CL). Therefore, identifying therapeutic targets that break this vicious cycle may offer novel promising therapeutic interventions for the cure of glomerular diseases.

AUTHOR CONTRIBUTIONS

MG and FF conceived and wrote the manuscript. SM and AF critically reviewed and improved the entire manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

AF and SM are supported by NIH grants R01DK117599, R01DK104753, and R01CA227493. AF is also supported by U54DK083912, UM1DK100846, U01DK116101, and UL1TR000460 (Miami Clinical Translational Science Institute). FF is supported by Department of Defense (DoD) Discovery Award (PR180598). MG is supported by a predoctoral fellowship of the American Heart Association (18PRE34030042).

ACKNOWLEDGMENTS

We would like to thank the Katz family for supporting this study. Figures were created with BioRender.

REFERENCES

Abe, Y., Sakairi, T., Kajiyama, H., Shrivastav, S., Beeson, C., and Kopp, J. B. (2010). Bioenergetic characterization of mouse podocytes. *Am. J. Physiol. Cell Physiol.* 299, C464–C476. doi: 10.1152/ajpcell.00563.2009

Agarwal, R. (2006). Effects of statins on renal function. *Am. J. Cardiol.* 97, 748–755. doi: 10.1016/j.amjcard.2005.09.110

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., et al. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290, 457–465. doi: 10.1038/290457a0

Aon, M. A., Bhatt, N., and Cortassa, S. C. (2014). Mitochondrial and cellular mechanisms for managing lipid excess. *Front. Physiol.* 5:282. doi: 10.3389/fphys.2014.00282

Ashraf, S., Gee, H. Y., Woerner, S., Xie, L. X., Vega-Warner, V., Lovric, S., et al. (2013). ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. *J. Clin. Invest.* 123, 5179–5189. doi: 10.1172/JCI69000

Baud, L., and Ardaillou, R. (1993). Involvement of reactive oxygen species in kidney damage. *Br. Med. Bull.* 49, 621–629. doi: 10.1093/oxfordjournals.bmb.a072635

Becher, M. W., Wills, M. L., Noll, W. W., Hurko, O., and Price, D. L. (1999). Kearns-Sayre syndrome with features of Pearson's marrow-pancreas syndrome and a novel 2905-base pair mitochondrial DNA deletion. *Hum. Pathol.* 30, 577–581. doi: 10.1016/S0046-8177(99)90204-6

Bhargava, P., and Schnellmann, R. G. (2017). Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.* 13, 629–646. doi: 10.1038/nrneph.2017.107

Blanco, S., Vaquero, M., Gomez-Guerrero, C., Lopez, D., Egido, J., and Romero, R. (2005). Potential role of angiotensin-converting enzyme inhibitors and statins on early podocyte damage in a model of type 2 diabetes mellitus, obesity, and mild hypertension. *Am. J. Hypertens.* 18, 557–565. doi: 10.1016/j.amjhyper.2004.10.034

Boren, J., and Brindle, K. M. (2012). Apoptosis-induced mitochondrial dysfunction causes cytoplasmic lipid droplet formation. *Cell Death Differ.* 19, 1561–1570. doi: 10.1038/cdd.2012.34

Borysiewicz, L. K., Soutar, A. K., Evans, D. J., Thompson, G. R., and Rees, A. J. (1982). Renal failure in familial lecithin: cholesterol acyltransferase deficiency. *Q. J. Med.* 51, 411–426.

Buja, L. M., Kovanen, P. T., and Bilheimer, D. W. (1979). Cellular pathology of homozygous familial hypercholesterolemia. *Am. J. Pathol.* 97, 327–357.

Bussolati, B., Deregibus, M. C., Fonsato, V., Doublier, S., Spatola, T., Procida, S., et al. (2005). Statins prevent oxidized LDL-induced injury of glomerular podocytes by activating the phosphatidylinositol 3-kinase/AKT-signaling pathway. *J. Am. Soc. Nephrol.* 16, 1936–1947. doi: 10.1681/ASN.2004080629

Calkin, A. C., Giunti, S., Jandeleit-Dahm, K. A., Allen, T. J., Cooper, M. E., and Thomas, M. C. (2006). PPAR-alpha and -gamma agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mouse. *Nephrol. Dial. Transplant.* 21, 2399–2405. doi: 10.1093/ndt/gfl212

Calvo, S. E., Clauser, K. R., and Mootha, V. K. (2016). MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res.* 44, D1251–D1257. doi: 10.1093/nar/gkv1003

Che, R., Yuan, Y., Huang, S., and Zhang, A. (2014). Mitochondrial dysfunction in the pathophysiology of renal diseases. *Am. J. Physiol. Renal Physiol.* 306, F367–F378. doi: 10.1152/ajprenal.00571.2013

Cheong, H. I., Chae, J. H., Kim, J. S., Park, H. W., Ha, I. S., Hwang, Y. S., et al. (1999). Hereditary glomerulopathy associated with a mitochondrial tRNA(Leu) gene mutation. *Pediatr. Nephrol.* 13, 477–480. doi: 10.1007/s004670050641

Claypool, S. M., and Koehler, C. L. (2012). The complexity of cardiolipin in health and disease. *Trends Biochem. Sci.* 37, 32–41. doi: 10.1016/j.tibs.2011.09.003

Czajka, A., and Malik, A. N. (2016). Hyperglycemia induced damage to mitochondrial respiration in renal mesangial and tubular cells: implications for diabetic nephropathy. *Redox Biol.* 10, 100–107. doi: 10.1016/j.redox.2016.09.007

Daehn, I., Casalena, G., Zhang, T., Shi, S., Fenninger, F., Barasch, N., et al. (2014). Endothelial mitochondrial oxidative stress determines podocyte depletion in segmental glomerulosclerosis. *J. Clin. Invest.* 124, 1608–1621. doi: 10.1172/JCI71195

D'Agati, V. D., Kaskel, F. J., and Falk, R. J. (2011). Focal segmental glomerulosclerosis. *N. Engl. J. Med.* 365, 2398–2411. doi: 10.1056/NEJMra1106556

Decleves, A. E., Mathew, A. V., Cunard, R., and Sharma, K. (2011). AMPK mediates the initiation of kidney disease induced by a high-fat diet. *J. Am. Soc. Nephrol.* 22, 1846–1855. doi: 10.1681/ASN.2011010026

Decleves, A. E., Zolkipli, Z., Satriano, J., Wang, L., Nakayama, T., Rogac, M., et al. (2014). Regulation of lipid accumulation by AMP-activated kinase [corrected] in high fat diet-induced kidney injury. *Kidney Int.* 85, 611–623. doi: 10.1038/ki.2013.462

Desbats, M. A., Lunardi, G., Doimo, M., Trevisson, E., and Salviati, L. (2015). Genetic bases and clinical manifestations of coenzyme Q10 (CoQ 10) deficiency. *J. Inherit. Metab. Dis.* 38, 145–156. doi: 10.1007/s10545-014-9749-9

Di Donato, S. (2009). Multisystem manifestations of mitochondrial disorders. *J. Neurol.* 256, 693–710. doi: 10.1007/s00415-009-5028-3

Ding, Z., Liu, S., Wang, X., Deng, X., Fan, Y., Sun, C., et al. (2015). Hemodynamic shear stress via ROS modulates PCSK9 expression in human vascular endothelial and smooth muscle cells and along the mouse aorta. *Antioxid. Redox Signal.* 22, 760–771. doi: 10.1089/ars.2014.6054

Ding, Z., Liu, S., Wang, X., Mathur, P., Dai, Y., Theus, S., et al. (2016). Cross-talk between PCSK9 and damaged mtDNA in vascular smooth muscle cells: role in apoptosis. *Antioxid. Redox Signal.* 25, 997–1008. doi: 10.1089/ars.2016.6631

Ducasa, G. M., Mitrofanova, A., and Fornoni, A. (2019a). Crosstalk between lipids and mitochondria in diabetic kidney disease. *Curr. Diab. Rep.* 19:144. doi: 10.1007/s11892-019-1263-x

Ducasa, G. M., Mitrofanova, A., Mallela, S. K., Liu, X., Molina, J., Sloan, A., et al. (2019b). ATP-binding cassette A1 deficiency causes cardiolipin-driven mitochondrial dysfunction in podocytes. *J. Clin. Invest.* 129, 3387–3400. doi: 10.1172/JCI125316

Dugan, L. L., You, Y. H., Ali, S. S., Diamond-Stanic, M., Miyamoto, S., DeCleves, A. E., et al. (2013). AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. *J. Clin. Invest.* 123, 4888–4899. doi: 10.1172/JCI66218

Edwards, P. A., Tabor, D., Kast, H. R., and Venkateswaran, A. (2000). Regulation of gene expression by SREBP and SCAP. *Biochim. Biophys. Acta* 1529, 103–113. doi: 10.1016/s1388-1981(00)00140-2

Eid, N., Ito, Y., Maemura, K., and Otsuki, Y. (2013). Elevated autophagic sequestration of mitochondria and lipid droplets in steatotic hepatocytes of chronic ethanol-treated rats: an immunohistochemical and electron microscopic study. *J. Mol. Histol.* 44, 311–326. doi: 10.1007/s10735-013-9483-x

Elmaci, A. M., Peru, H., Akin, F., Akcoren, Z., Caglar, M., and Ozel, A. (2007). A case of homozygous familial hypercholesterolemia with focal segmental glomerulosclerosis. *Pediatr. Nephrol.* 22, 1803–1805. doi: 10.1007/s00467-007-0534-y

Emanuelsson, F., Nordestgaard, B. G., and Benn, M. (2018). Familial hypercholesterolemia and risk of peripheral arterial disease and chronic kidney disease. *J. Clin. Endocrinol. Metab.* 103, 4491–4500. doi: 10.1210/jc.2018-01058

Eom, M., Hudkins, K. L., and Alpers, C. E. (2015). Foam cells and the pathogenesis of kidney disease. *Curr. Opin. Nephrol. Hypertens.* 24, 245–251. doi: 10.1097/MNH.0000000000000112

Ernster, L., and Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* 1271, 195–204. doi: 10.1016/0925-4439(95)00028-3

Ferrans, V. J., and Fredrickson, D. S. (1975). The pathology of Tangier disease. A light and electron microscopic study. *Am. J. Pathol.* 78, 101–158.

Fink, B. D., Herlein, J. A., O'Malley, Y., and Sivitz, W. I. (2012). Endothelial cell and platelet bioenergetics: effect of glucose and nutrient composition. *PLoS One* 7:e39430. doi: 10.1371/journal.pone.0039430

Finsterer, J., and Scorza, F. A. (2017). Renal manifestations of primary mitochondrial disorders. *Biomed. Rep.* 6, 487–494. doi: 10.3892/br.2017.892

Fobker, M., Voss, R., Reinecke, H., Crone, C., Assmann, G., and Walter, M. (2001). Accumulation of cardiolipin and lysocardiolipin in fibroblasts from Tangier disease subjects. *FEBS Lett.* 500, 157–162. doi: 10.1016/S0014-5793(01)02578-9

Forbes, J. M., and Thorburn, D. R. (2018). Mitochondrial dysfunction in diabetic kidney disease. *Nat. Rev. Nephrol.* 14, 291–312. doi: 10.1038/nrneph.2018.9

Fornoni, A., Merscher, S., and Kopp, J. B. (2014). Lipid biology of the podocyte--new perspectives offer new opportunities. *Nat. Rev. Nephrol.* 10, 379–388. doi: 10.1038/nrneph.2014.87

Gai, Z., Gui, T., Hiller, C., and Kullak-Ublick, G. A. (2016). Farnesoid X receptor protects against kidney injury in uninephrectomized obese mice. *J. Biol. Chem.* 291, 2397–2411. doi: 10.1074/jbc.M115.694323

Gai, Z., Wang, T., Visentin, M., Kullak-Ublick, G. A., Fu, X., and Wang, Z. (2019). Lipid accumulation and chronic kidney disease. *Nutrients* 11:722. doi: 10.3390/nu11040722

Gasser, D. L., Winkler, C. A., Peng, M., An, P., McKenzie, L. M., Kirk, G. D., et al. (2013). Focal segmental glomerulosclerosis is associated with a PDSS2 haplotype and, independently, with a decreased content of coenzyme Q10. *Am. J. Physiol. Renal Physiol.* 305, F1228–F1238. doi: 10.1152/ajprenal.00143.2013

Grafft, C. A., Fervenza, F. C., Semret, M. H., Orloff, S., and Sethi, S. (2009). Renal involvement in Neimann-Pick disease. *NDT Plus* 2, 448–451. doi: 10.1093/ndtplus/sfp101

Grone, H. J., Walli, A. K., Grone, E., Kramer, A., Clemens, M. R., and Seidel, D. (1990). Receptor mediated uptake of apo B and apo E rich lipoproteins by human glomerular epithelial cells. *Kidney Int.* 37, 1449–1459. doi: 10.1038/ki.1990.135

Gucer, S., Talim, B., Asan, E., Korkusuz, P., Ozen, S., Unal, S., et al. (2005). Focal segmental glomerulosclerosis associated with mitochondrial cytopathy: report of two cases with special emphasis on podocytes. *Pediatr. Dev. Pathol.* 8, 710–717. doi: 10.1007/s10024-005-0058-z

Gutwein, P., Abdel-Bakky, M. S., Doberstein, K., Schramme, A., Beckmann, J., Schaefer, L., et al. (2009a). CXCL16 and oxLDL are induced in the onset of diabetic nephropathy. *J. Cell. Mol. Med.* 13, 3809–3825. doi: 10.1111/j.1582-4934.2009.00761.x

Gutwein, P., Abdel-Bakky, M. S., Schramme, A., Doberstein, K., Kampfer-Kolb, N., Amann, K., et al. (2009b). CXCL16 is expressed in podocytes and acts as a scavenger receptor for oxidized low-density lipoprotein. *Am. J. Pathol.* 174, 2061–2072. doi: 10.2353/ajpath.2009.080960

Haas, M. E., Levenson, A. E., Sun, X., Liao, W. H., Rutkowski, J. M., de Ferranti, S. D., et al. (2016). The role of proprotein convertase subtilisin/kexin type 9 in nephrotic syndrome-associated hypercholesterolemia. *Circulation* 134, 61–72. doi: 10.1161/CIRCULATIONAHA.115.020912

Hallan, S., Afkarian, M., Zelnick, L. R., Kestenbaum, B., Sharma, S., Saito, R., et al. (2017). Metabolomics and gene expression analysis reveal down-regulation of the citric acid (TCA) cycle in non-diabetic CKD patients. *EBioMedicine* 26, 68–77. doi: 10.1016/j.ebiom.2017.10.027

Hansell, P., Welch, W. J., Blantz, R. C., and Palm, F. (2013). Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. *Clin. Exp. Pharmacol. Physiol.* 40, 123–137. doi: 10.1111/1440-1681.12034

Hardie, D. G., Ross, F. A., and Hawley, S. A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* 13, 251–262. doi: 10.1038/nrm3311

Heeringa, S. F., Chernin, G., Chaki, M., Zhou, W., Sloan, A. J., Ji, Z., et al. (2011). COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J. Clin. Invest.* 121, 2013–2024. doi: 10.1172/JCI45693

Heinz, L. X., Baumann, C. L., Koberlin, M. S., Snijder, B., Gawish, R., Shui, G., et al. (2015). The lipid-modifying enzyme SMPDL3B negatively regulates innate immunity. *Cell Rep.* 11, 1919–1928. doi: 10.1016/j.celrep.2015.05.006

Herman-Edelstein, M., Scherzer, P., Tobar, A., Levi, M., and Gafter, U. (2014). Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy. *J. Lipid Res.* 55, 561–572. doi: 10.1194/jlr.P040501

Heymann, W., Hackel, D. B., Harwood, S., Wilson, S. G., and Hunter, J. L. (1959). Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc. Soc. Exp. Biol. Med.* 100, 660–664. doi: 10.3181/00379727-100-24736

Hodgin, J. B., Nair, V., Zhang, H., Randolph, A., Harris, R. C., Nelson, R. G., et al. (2013). Identification of cross-species shared transcriptional networks of diabetic nephropathy in human and mouse glomeruli. *Diabetes* 62, 299–308. doi: 10.2337/db11-1667

Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* 109, 1125–1131. doi: 10.1172/JCI0215593

Hotta, O., Inoue, C. N., Miyabayashi, S., Furuta, T., Takeuchi, A., and Taguma, Y. (2001). Clinical and pathologic features of focal segmental glomerulosclerosis with mitochondrial tRNALeu(UUR) gene mutation. *Kidney Int.* 59, 1236–1243. doi: 10.1046/j.1523-1755.2001.0590041236.x

Idrovo, J. P., Yang, W. L., Nicastro, J., Coppa, G. F., and Wang, P. (2012). Stimulation of carnitine palmitoyltransferase 1 improves renal function and attenuates tissue damage after ischemia/reperfusion. *J. Surg. Res.* 177, 157–164. doi: 10.1016/j.jss.2012.05.053

Imasawa, T., Obre, E., Bellance, N., Lavie, J., Imasawa, T., Rigothier, C., et al. (2017). High glucose repatterns human podocyte energy metabolism during differentiation and diabetic nephropathy. *FASEB J.* 31, 294–307. doi: 10.1096/fj.201600293R

Jeong, H. J., Lee, H. S., Kim, K. S., Kim, Y. K., Yoon, D., and Park, S. W. (2008). Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J. Lipid Res.* 49, 399–409. doi: 10.1194/jlr.M700443-JLR200

Jiang, T., Wang, Z., Proctor, G., Moskowitz, S., Liebman, S. E., Rogers, T., et al. (2005). Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. *J. Biol. Chem.* 280, 32317–32325. doi: 10.1074/jbc.M500801200

Kagan, V. E., Tyurin, V. A., Jiang, J., Tyurina, Y. Y., Ritov, V. B., Amoscato, A. A., et al. (2005). Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat. Chem. Biol.* 1, 223–232. doi: 10.1038/nchembio727

Kang, H. M., Ahn, S. H., Choi, P., Ko, Y. A., Han, S. H., Chinga, F., et al. (2015). Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat. Med.* 21, 37–46. doi: 10.1038/nm.3762

Kawakami, T., Gomez, I. G., Ren, S., Hudkins, K., Roach, A., Alpers, C. E., et al. (2015). Deficient autophagy results in mitochondrial dysfunction and FSGS. *J. Am. Soc. Nephrol.* 26, 1040–1052. doi: 10.1681/ASN.2013111202

Ke, Q., Yuan, Q., Qin, N., Shi, C., Luo, J., Fang, Y., et al. (2020). UCP2-induced hypoxia promotes lipid accumulation and tubulointerstitial fibrosis during ischemic kidney injury. *Cell Death Dis.* 11:26. doi: 10.1038/s41419-019-2219-4

Keech, A., Simes, R. J., Barter, P., Best, J., Scott, R., Taskinen, M. R., et al. (2005). Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 366, 1849–1861. doi: 10.1016/S0140-6736(05)67667-2

Kerjaschki, D., Exner, M., Ullrich, R., Susani, M., Curtiss, L. K., Witztum, J. L., et al. (1997). Pathogenic antibodies inhibit the binding of apolipoproteins to megalin/gp330 in passive Heymann nephritis. *J. Clin. Invest.* 100, 2303–2309. doi: 10.1172/JCI119768

Kriz, W., Gretz, N., and Lemley, K. V. (1998). Progression of glomerular diseases: is the podocyte the culprit? *Kidney Int.* 54, 687–697. doi: 10.1046/j.1523-1755.1998.00044.x

Kruger, C., Burke, S. J., Collier, J. J., Nguyen, T. T., Salbaum, J. M., and Stadler, K. (2018). Lipid peroxidation regulates podocyte migration and cytoskeletal structure through redox sensitive RhoA signaling. *Redox Biol.* 16, 248–254. doi: 10.1016/j.redox.2018.02.024

Kurogouchi, F., Oguchi, T., Mawatari, E., Yamaura, S., Hora, K., Takei, M., et al. (1998). A case of mitochondrial cytopathy with a typical point mutation for MELAS, presenting with severe focal-segmental glomerulosclerosis as main clinical manifestation. *Am. J. Nephrol.* 18, 551–556. doi: 10.1159/000013406

Lackner, L. (2014). Shaping the dynamic mitochondrial network. *BMC Biol.* 12:35. doi: 10.1186/1741-7007-1112-1135

Lambert, G., Sakai, N., Vaisman, B. L., Neufeld, E. B., Marteyn, B., Chan, C. C., et al. (2001). Analysis of glomerulosclerosis and atherosclerosis in lecithin cholesterol acyltransferase-deficient mice. *J. Biol. Chem.* 276, 15090–15098. doi: 10.1074/jbc.M008466200

Lee, E., Choi, J., and Lee, H. S. (2017). Palmitate induces mitochondrial superoxide generation and activates AMPK in podocytes. *J. Cell. Physiol.* 232, 3209–3217. doi: 10.1002/jcp.25867

Lee, E., and Lee, H. S. (2018). Peroxidase expression is decreased by palmitate in cultured podocytes but increased in podocytes of advanced diabetic nephropathy. *J. Cell. Physiol.* 233, 9060–9069. doi: 10.1002/jcp.26875

Lenaz, G., and Genova, M. L. (2010). Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. *Antioxid. Redox Signal.* 12, 961–1008. doi: 10.1089/ars.2009.2704

Li, S. Y., Park, J., Qiu, C., Han, S. H., Palmer, M. B., Arany, Z., et al. (2017). Increasing the level of peroxisome proliferator-activated receptor gamma coactivator-1alpha in podocytes results in collapsing glomerulopathy. *JCI Insight* 2:e92930. doi: 10.1172/jci.insight.92930

Lin, C. Y., Lee, T. S., Chen, C. C., Chang, C. A., Lin, Y. J., Hsu, Y. P., et al. (2011). Endothelin-1 exacerbates lipid accumulation by increasing the protein degradation of the ATP-binding cassette transporter G1 in macrophages. *J. Cell. Physiol.* 226, 2198–2205. doi: 10.1002/jcp.22556

Liu, T., Chen, X. M., Sun, J. Y., Jiang, X. S., Wu, Y., Yang, S., et al. (2018). Palmitic acid-induced podocyte apoptosis via the reactive oxygen species-dependent mitochondrial pathway. *Kidney Blood Press. Res.* 43, 206–219. doi: 10.1159/000487673

Lopez, L. C., Schuelke, M., Quinzii, C. M., Kanki, T., Rodenburg, R. J., Naini, A., et al. (2006). Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. *Am. J. Hum. Genet.* 79, 1125–1129. doi: 10.1086/510023

Lovric, S., Ashraf, S., Tan, W., and Hildebrandt, F. (2016). Genetic testing in steroid-resistant nephrotic syndrome: when and how? *Nephrol. Dial. Transplant.* 31, 1802–1813. doi: 10.1093/ndt/gfv355

Martinez-Garcia, C., Izquierdo, A., Velagapudi, V., Vivas, Y., Velasco, I., Campbell, M., et al. (2012). Accelerated renal disease is associated with the development of metabolic syndrome in a glucolipotoxic mouse model. *Dis. Model. Mech.* 5, 636–648. doi: 10.1242/dmm.009266

Martinez-Garcia, C., Izquierdo-Lahuerta, A., Vivas, Y., Velasco, I., Yeo, T. K., Chen, S., et al. (2015). Renal lipotoxicity-associated inflammation and insulin resistance affects actin cytoskeleton organization in podocytes. *PLoS One* 10:e0142291. doi: 10.1371/journal.pone.0142291

Merscher-Gomez, S., Guzman, J., Pedigo, C. E., Lehto, M., Aguillon-Prada, R., Mendez, A., et al. (2013). Cyclodextrin protects podocytes in diabetic kidney disease. *Diabetes* 62, 3817–3827. doi: 10.2337/db13-0399

Meulemans, A., Seneca, S., Lagae, L., Lissens, W., De Paepe, B., Smet, J., et al. (2006). A novel mitochondrial transfer RNA(Asn) mutation causing multiorgan failure. *Arch. Neurol.* 63, 1194–1198. doi: 10.1001/archneur.63.8.1194

Mitrofanova, A., Molina, J., Varona Santos, J., Guzman, J., Morales, X. A., Ducasa, G. M., et al. (2018). Hydroxypropyl-beta-cyclodextrin protects from kidney disease in experimental alport syndrome and focal segmental glomerulosclerosis. *Kidney Int.* 94, 1151–1159. doi: 10.1016/j.kint.2018.06.031

Narumi, K., Mishima, E., Akiyama, Y., Matsuhashi, T., Nakamichi, T., Kisu, K., et al. (2018). Focal segmental glomerulosclerosis associated with chronic progressive external ophthalmoplegia and mitochondrial DNA A3243G mutation. *Nephron* 138, 243–248. doi: 10.1159/000485109

Nolasco, F., Cameron, J. S., Reuben, R., Hartley, B., Carvalho, F., and Coelho, R. A. (1985). Interstitial foam cells in the nephrotic syndrome belong to the monocyte/macrophage lineage. *Proc. Eur. Dial. Transplant. Assoc. Eur. Ren. Assoc.* 21, 666–671.

Ossoli, A., Lucca, F., Boscutti, G., Remaley, A. T., and Calabresi, L. (2015). Familial LCAT deficiency: from pathology to enzyme replacement therapy. *J. Clin. Lipidol.* 10, 405–413. doi: 10.2217/clp.15.34

Owen, O., Kalhan, S., and Hanson, R. (2002). The key role of anaplerosis and cataplerosis for citric acid cycle function. *J. Biol. Chem.* 277, 30409–30412. doi: 10.1074/jbc.R200006200

Palomer, X., Pizarro-Delgado, J., Barroso, E., and Vazquez-Carrera, M. (2018). Palmitic and oleic acid: the yin and yang of fatty acids in type 2 diabetes mellitus. *Trends Endocrinol. Metab.* 29, 178–190. doi: 10.1016/j.tem.2017.11.009

Pedigo, C. E., Ducasa, G. M., Leclercq, F., Sloan, A., Mitrofanova, A., Hashmi, T., et al. (2016). Local TNF causes NFATc1-dependent cholesterol-mediated podocyte injury. *J. Clin. Invest.* 126, 3336–3350. doi: 10.1172/JCI85939

Pfeiffer, K., Gohil, V., Stuart, R. A., Hunte, C., Brandt, U., Greenberg, M. L., et al. (2003). Cardiolipin stabilizes respiratory chain supercomplexes. *J. Biol. Chem.* 278, 52873–52880. doi: 10.1074/jbc.M308366200

Picard, M., Jung, B., Liang, F., Azuelos, I., Hussain, S., Goldberg, P., et al. (2012). Mitochondrial dysfunction and lipid accumulation in the human diaphragm during mechanical ventilation. *Am. J. Respir. Crit. Care Med.* 186, 1140–1149. doi: 10.1164/rccm.201206-0982OC

Qi, H., Casalena, G., Shi, S., Yu, L., Ebefors, K., Sun, Y., et al. (2017). Glomerular endothelial mitochondrial dysfunction is essential and characteristic of diabetic kidney disease susceptibility. *Diabetes* 66, 763–778. doi: 10.2337/db16-0695

Rinschen, M. M., Palygin, O., Guijas, C., Palermo, A., Palacio-Escat, N., Domingo-Almenara, X., et al. (2019). Metabolic rewiring of the hypertensive kidney. *Sci. Signal.* 12:eaax9760. doi: 10.1126/scisignal.aax9760

Rong, S., Cortes, V. A., Rashid, S., Anderson, N. N., McDonald, J. G., Liang, G., et al. (2017). Expression of SREBP-1c requires SREBP-2-mediated generation of a sterol ligand for LXR in livers of mice. *elife* 6:e25015. doi: 10.7554/eLife.25015

Rousset, S., Alves-Guerra, M. C., Mozo, J., Miroux, B., Cassard-Doulcier, A. M., Bouillaud, F., et al. (2004). The biology of mitochondrial uncoupling proteins. *Diabetes* 53(Suppl. 1), S130–S135. doi: 10.2337/diabetes.53.2007.S130

Ruan, X. Z., Moorhead, J. F., Fernando, R., Wheeler, D. C., Powis, S. H., and Varghese, Z. (2003). PPAR agonists protect mesangial cells from interleukin 1beta-induced intracellular lipid accumulation by activating the ABCA1 cholesterol efflux pathway. *J. Am. Soc. Nephrol.* 14, 593–600. doi: 10.1097/01.asn.0000050414.52908.da

Ruan, X. Z., Varghese, Z., and Moorhead, J. F. (2009). An update on the lipid nephrotoxicity hypothesis. *Nat. Rev. Nephrol.* 5, 713–721. doi: 10.1038/nrneph.2009.184

Rudnicki, M., Mayr, J. A., Zschocke, J., Antretter, H., Regele, H., Feichtinger, R. G., et al. (2016). MELAS syndrome and kidney disease without fanconi syndrome or proteinuria: a case report. *Am. J. Kidney Dis.* 68, 949–953. doi: 10.1053/j.ajkd.2016.06.027

Saiki, R., Lunceford, A. L., Shi, Y., Marbois, B., King, R., Pachuski, J., et al. (2008). Coenzyme Q10 supplementation rescues renal disease in Pdss2kd/kd mice with mutations in prenyl diphosphate synthase subunit 2. *Am. J. Physiol. Renal Physiol.* 295, F1535–F1544. doi: 10.1152/ajprenal.90445.2008

Saiki, R., Nagata, A., Kainou, T., Matsuda, H., and Kawamukai, M. (2005). Characterization of solanesyl and decaprenyl diphosphate synthases in mice and humans. *FEBS J.* 272, 5606–5622. doi: 10.1111/j.1742-4658.2005.04956.x

Sam, R., Wu, H., Yue, L., Mazzone, T., Schwartz, M. M., Arruda, J. A., et al. (2006). Lipoprotein glomerulopathy: a new apolipoprotein E mutation with enhanced glomerular binding. *Am. J. Kidney Dis.* 47, 539–548. doi: 10.1053/j.ajkd.2005.12.031

Sato, S., Kitamura, H., Adachi, A., Sasaki, Y., and Ghazizadeh, M. (2006). Two types of autophagy in the podocytes in renal biopsy specimens: ultrastructural study. *J. Submicrosc. Cytol. Pathol.* 38, 167–174.

Scaglia, F., Vogel, H., Hawkins, E. P., Vladutiu, G. D., Liu, L. L., and Wong, L. J. (2003). Novel homoplasmic mutation in the mitochondrial tRNATyr gene associated with atypical mitochondrial cytopathy presenting with focal segmental glomerulosclerosis. *Am. J. Med. Genet. A* 123A, 172–178. doi: 10.1002/ajmg.a.20315

Schaefer, E. J., Santos, R. D., and Asztalos, B. F. (2010). Marked HDL deficiency and premature coronary heart disease. *Curr. Opin. Lipidol.* 21, 289–297. doi: 10.1097/MOL.0b013e32833c1ef6

Schmit, D., Fliser, D., and Speer, T. (2019). Proprotein convertase subtilisin/kexin type 9 in kidney disease. *Nephrol. Dial. Transplant.* 34, 1266–1271. doi: 10.1093/ndt/gfz122

Schrauwen-Hinderling, V. B., Kooi, M. E., and Schrauwen, P. (2016). Mitochondrial function and diabetes: consequences for skeletal and cardiac muscle metabolism. *Antioxid. Redox Signal.* 24, 39–51. doi: 10.1089/ars.2015.6291

Serezani, C. H., Kane, S., Medeiros, A. I., Cornett, A. M., Kim, S. H., Marques, M. M., et al. (2012). PTEN directly activates the actin depolymerization factor cofilin-1 during PGE2-mediated inhibition of phagocytosis of fungi. *Sci. Signal.* 5:ra12. doi: 10.1126/scisignal.2002448

Shi, Y., Wang, C., Zhou, X., Li, Y., Ma, Y., Zhang, R., et al. (2019). Downregulation of PTEN promotes podocyte endocytosis of lipids aggravating obesity-related glomerulopathy. *Am. J. Physiol. Renal Physiol.* 318, F589–F599. doi: 10.1152/ajprenal.00392.2019

Shrestha, P., van de Sluis, B., Dullaart, R. P. F., and van den Born, J. (2019). Novel aspects of PCSK9 and lipoprotein receptors in renal disease-related dyslipidemia. *Cell. Signal.* 55, 53–64. doi: 10.1016/j.cellsig.2018.12.001

Simons, M., Saffrich, R., Reiser, J., and Mundel, P. (1999). Directed membrane transport is involved in process formation in cultured podocytes. *J. Am. Soc. Nephrol.* 10, 1633–1639.

Simons, M., Schwarz, K., Kriz, W., Miettinen, A., Reiser, J., Mundel, P., et al. (2001). Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. *Am. J. Pathol.* 159, 1069–1077. doi: 10.1016/S0002-9440(10)61782-8

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., et al. (2009). Autophagy regulates lipid metabolism. *Nature* 458, 1131–1135. doi: 10.1038/nature07976

Sourris, K. C., Harcourt, B. E., Tang, P. H., Morley, A. L., Huynh, K., Penfold, S. A., et al. (2012). Ubiquinone (coenzyme Q10) prevents renal mitochondrial dysfunction in an experimental model of type 2 diabetes. *Free Radic. Biol. Med.* 52, 716–723. doi: 10.1016/j.freeradbiomed.2011.11.017

Spiegelman, B. M. (2007). Transcriptional control of mitochondrial energy metabolism through the PGC1 coactivators. *Novartis Found. Symp.* 287, 60–63.

Starr, M. C., Chang, I. J., Finn, L. S., Sun, A., Larson, A. A., Goebel, J., et al. (2018). COQ2 nephropathy: a treatable cause of nephrotic syndrome in children. *Pediatr. Nephrol.* 33, 1257–1261. doi: 10.1007/s00467-018-3937-z

Steinberg, G. R., Michell, B. J., van Denderen, B. J., Watt, M. J., Carey, A. L., Fam, B. C., et al. (2006). Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metab.* 4, 465–474. doi: 10.1016/j.cmet.2006.11.005

Stokes, M. B., Valeri, A. M., Markowitz, G. S., and D'Agati, V. D. (2006). Cellular focal segmental glomerulosclerosis: clinical and pathologic features. *Kidney Int.* 70, 1783–1792. doi: 10.1038/sj.ki.5001903

Stowe, D. F., and Camara, A. K. (2009). Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. *Antioxid. Redox Signal.* 11, 1373–1414. doi: 10.1089/ars.2008.2331

Sun, L., Halaihel, N., Zhang, W., Rogers, T., and Levi, M. (2002). Role of sterol regulatory element-binding protein 1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus. *J. Biol. Chem.* 277, 18919–18927. doi: 10.1074/jbc.M110650200

Szeto, H. H. (2014). First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br. J. Pharmacol.* 171, 2029–2050. doi: 10.1111/bph.12461

Szeto, H. H., and Birk, A. V. (2014). Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. *Clin. Pharmacol. Ther.* 96, 672–683. doi: 10.1038/clpt.2014.174

Szeto, H. H., Liu, S., Soong, Y., Alam, N., Prusky, G. T., and Seshan, S. V. (2016). Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. *Kidney Int.* 90, 997–1011. doi: 10.1016/j.kint.2016.06.013

Tanaka, Y., Kume, S., Araki, S., Isshiki, K., Chin-Kanasaki, M., Sakaguchi, M., et al. (2011). Fenofibrate, a PPARalpha agonist, has renoprotective effects in mice by enhancing renal lipolysis. *Kidney Int.* 79, 871–882. doi: 10.1038/ki.2010.530

Taniike, M., Fukushima, H., Yanagihara, I., Tsukamoto, H., Tanaka, J., Fujimura, H., et al. (1992). Mitochondrial tRNA(Ile) mutation in fatal cardiomyopathy. *Biochem. Biophys. Res. Commun.* 186, 47–53. doi: 10.1016/S0006-291X(05)80773-9

Tsimihodimos, V., and Elisaf, M. (2011). Lipoprotein glomerulopathy. *Curr. Opin. Lipidol.* 22, 262–269. doi: 10.1097/MOL.0b013e328345ebb0

Turnbull, D. M., and Rustin, P. (2016). Genetic and biochemical intricacy shapes mitochondrial cytopathies. *Neurobiol. Dis.* 92, 55–63. doi: 10.1016/j.nbd.2015.02.003

Vafai, S. B., and Mootha, V. K. (2012). Mitochondrial disorders as windows into an ancient organelle. *Nature* 491, 374–383. doi: 10.1038/nature11707

Vankoningsloo, S., Piens, M., Lecocq, C., Gilson, A., De Pauw, A., Renard, P., et al. (2005). Mitochondrial dysfunction induces triglyceride accumulation in 3T3-L1 cells: role of fatty acid beta-oxidation and glucose. *J. Lipid Res.* 46, 1133–1149. doi: 10.1194/jlr.M400464-JLR200

Vasta, V., Merritt, J. L. 2nd, Saneto, R. P., and Hahn, S. H. (2012). Next-generation sequencing for mitochondrial diseases: a wide diagnostic spectrum. *Pediatr. Int.* 54, 585–601. doi: 10.1111/j.1442-200X.2012.03644.x

Vincent, A. E., Ng, Y. S., White, K., Davey, T., Mannella, C., Falkous, G., et al. (2016). The spectrum of mitochondrial ultrastructural defects in mitochondrial myopathy. *Sci. Rep.* 6:30610. doi: 10.1038/srep30610

Wang, X. X., Jiang, T., Shen, Y., Adorini, L., Pruzanski, M., Gonzalez, F. J., et al. (2009). The farnesoid X receptor modulates renal lipid metabolism and diet-induced renal inflammation, fibrosis, and proteinuria. *Am. J. Physiol. Renal Physiol.* 297, F1587–F1596. doi: 10.1152/ajprenal.00404.2009

Wang, X. X., Luo, Y., Wang, D., Adorini, L., Pruzanski, M., Dobrinskikh, E., et al. (2017). A dual agonist of farnesoid X receptor (FXR) and the G protein-coupled receptor TGR5, INT-767, reverses age-related kidney disease in mice. *J. Biol. Chem.* 292, 12018–12024. doi: 10.1074/jbc.C117.794982

Wang, X. X., Wang, D., Luo, Y., Myakala, K., Dobrinskikh, E., Rosenberg, A. Z., et al. (2018). FXR/TGR5 dual agonist prevents progression of nephropathy in diabetes and obesity. *J. Am. Soc. Nephrol.* 29, 118–137. doi: 10.1681/ASN.2017020222

Wang, Z., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., et al. (2010). Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure. *Am. J. Clin. Nutr.* 92, 1369–1377. doi: 10.3945/ajcn.2010.29885

Widmeier, E., Yu, S., Nag, A., Chung, Y. W., Nakayama, M., Fernandez-Del-Rio, L., et al. (2020). ADCK4 deficiency destabilizes the coenzyme Q complex, which is rescued by 2,4-dihydroxybenzoic acid treatment. *J. Am. Soc. Nephrol.* 31, 1191–1211. doi: 10.1681/ASN.2019070756

Wu, Y., Chen, Y., Chen, D., Zeng, C., Li, L., and Liu, Z. (2009). Presence of foam cells in kidney interstitium is associated with progression of renal injury in patients with glomerular diseases. *Nephron Clin. Pract.* 113, c155–c161. doi: 10.1159/000232596

Xu, S., Nam, S. M., Kim, J. H., Das, R., Choi, S. K., Nguyen, T. T., et al. (2015). Palmitate induces ER calcium depletion and apoptosis in mouse podocytes subsequent to mitochondrial oxidative stress. *Cell Death Dis.* 6:e1976. doi: 10.1038/cddis.2015.331

Yang, J., Yang, Y., and Hu, Z. (2018). A novel ADCK4 mutation in a Chinese family with ADCK4-associated glomerulopathy. *Biochem. Biophys. Res. Commun.* 506, 444–449. doi: 10.1016/j.bbrc.2018.10.102

Yoo, T. H., Pedigo, C. E., Guzman, J., Correa-Medina, M., Wei, C., Villarreal, R., et al. (2015). Sphingomyelinase-like phosphodiesterase 3b expression levels determine podocyte injury phenotypes in glomerular disease. *J. Am. Soc. Nephrol.* 26, 133–147. doi: 10.1681/ASN.2013111213

Yu, X. H., Fu, Y. C., Zhang, D. W., Yin, K., and Tang, C. K. (2013). Foam cells in atherosclerosis. *Clin. Chim. Acta* 424, 245–252. doi: 10.1016/j.cca.2013.06.006

Yuan, Z., Cao, A., Liu, H., Guo, H., Zang, Y., Wang, Y., et al. (2017). Calcium uptake via mitochondrial uniporter contributes to palmitic acid-induced apoptosis in mouse podocytes. *J. Cell. Biochem.* 118, 2809–2818. doi: 10.1002/jcb.25930

Zhang, Y., Ma, K. L., Liu, J., Wu, Y., Hu, Z. B., Liu, L., et al. (2015a). Dysregulation of low-density lipoprotein receptor contributes to podocyte injuries in diabetic nephropathy. *Am. J. Physiol. Endocrinol. Metab.* 308, E1140–E1148. doi: 10.1152/ajpendo.00591.2014

Zhang, Y., Ma, K. L., Liu, J., Wu, Y., Hu, Z. B., Liu, L., et al. (2015b). Inflammatory stress exacerbates lipid accumulation and podocyte injuries in diabetic nephropathy. *Acta Diabetol.* 52, 1045–1056. doi: 10.1007/s00592-015-0753-9

Zhao, K., Zhao, G. M., Wu, D., Soong, Y., Birk, A. V., Schiller, P. W., et al. (2004). Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J. Biol. Chem.* 279, 34682–34690. doi: 10.1074/jbc.M402999200

Zheng, F., Fornoni, A., Elliot, S. J., Guan, Y., Breyer, M. D., Striker, L. J., et al. (2002). Upregulation of type I collagen by TGF-beta in mesangial cells is blocked by PPARgamma activation. *Am. J. Physiol. Renal Physiol.* 282, F639–F648. doi: 10.1152/ajprenal.00189.2001

Zhou, Y., Kong, X., Zhao, P., Yang, H., Chen, L., Miao, J., et al. (2011). Peroxisome proliferator-activated receptor-alpha is renoprotective in doxorubicin-induced glomerular injury. *Kidney Int.* 79, 1302–1311. doi: 10.1038/ki.2011.17

Zhou, X., Muise, E. S., Haimbach, R., Sebhat, I. K., Zhu, Y., Liu, F., et al. (2019). PAN-AMPK activation improves renal function in a rat model of progressive diabetic nephropathy. *J. Pharmacol. Exp. Ther.* 371, 45–55. doi: 10.1124/jpet.119.258244

Conflict of Interest: AF and SM are inventors on pending or issued patents (PCT/US2019/041730 and PCT/US2019/032215) aimed at diagnosing or treating proteinuric kidney diseases. They stand to gain royalties from the future commercialization of these patents. AF is vice-president of L&F Health LLC and is a consultant for ZyVersa Therapeutics, Inc. ZyVersa Therapeutics, Inc. has licensed worldwide rights to develop and commercialize hydroxypropyl-beta-cyclodextrin from L&F Research for the treatment of kidney disease. AF is founder of LipoNexT LLC. SM is a consultant for Kintai Therapeutics, Inc. and holds equity interest in L&F Research. AF and SM are supported by Hoffman-La Roche and by Boehringer Ingelheim.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 09 July 2020
doi: 10.3389/fphys.2020.00794

The Link Between the Mitochondrial Fatty Acid Oxidation Derangement and Kidney Injury

*Lara Console[1], Mariafrancesca Scalise[1], Nicola Giangregorio[2], Annamaria Tonazzi[2], Maria Barile[3] and Cesare Indiveri[1,2]**

[1] Unit of Biochemistry and Molecular Biotechnology, Department DiBEST (Biologia, Ecologia, Scienze della Terra), University of Calabria, Rende, Italy, [2] CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM), Bari, Italy, [3] Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari Aldo Moro, Bari, Italy

Renal proximal tubular cells are high energy-demanding cells mainly relying on fatty acid oxidation. In stress conditions, such as transient hypoxia, fatty acid oxidation (FAO) decreases and carbohydrate catabolism fails to compensate for the energy demand. In this scenario, the surviving tubular cells exhibit the peculiar phenotype associated with fibrosis that is the histological manifestation of a process culminating in chronic and end-stage kidney disease. Genome-wide transcriptome analysis revealed that, together with inflammation, FAO is the top dysregulated pathway in kidney diseases with a decreased expression of key FAO enzymes and regulators. Another evidence that links the derangement of FAO to fibrosis is the progressive decrease of the expression of peroxisome proliferator-activated receptor α (PPARα) in aged people, that triggers the age-associated renal fibrosis. To allow FAO completion, a coordinate network of enzymes and transport proteins is required. Indeed, the mitochondrial inner membrane is impermeable to fatty acyl-CoAs and a specialized system, well known as carnitine shuttle, is needed for translocating fatty acids moieties, conjugated with carnitine, into mitochondrial matrix for the β-oxidation. The first component of this system is the carnitine palmitoyltransferase 1 (CPT1) responsible for transfer acyl moieties to carnitine. Several studies indicated that the stimulation of CPT1 activity and expression has a protective effect against renal fibrosis. Therefore, the network of enzymes and transporters linked to FAO may represent potential pharmacological targets deserving further attention in the development of new drugs to attenuate renal dysfunction.

Keywords: mitochondria, β-oxidation, carnitine, kidney, CPT

OPEN ACCESS

Edited by:
Giuliano Ciarimboli, University of Münster, Germany

Reviewed by:
Katarzyna A. Nalecz, Nencki Institute of Experimental Biology (PAS), Poland
Carlos Palmeira, University of Coimbra, Portugal

****Correspondence:***
Cesare Indiveri
cesare.indiveri@unical.it

Specialty section:
This article was submitted to Mitochondrial Research, a section of the journal Frontiers in Physiology

Received: *14 May 2020*
Accepted: *15 June 2020*
Published: *09 July 2020*

Citation:
Console L, Scalise M, Giangregorio N, Tonazzi A, Barile M and Indiveri C (2020) The Link Between the Mitochondrial Fatty Acid Oxidation Derangement and Kidney Injury. Front. Physiol. 11:794. doi: 10.3389/fphys.2020.00794

INTRODUCTION

The kidney is one of the most energy-demanding organs in the human body, as demonstrated by Elia (Elia, 1992) in a study performed in 1992 describing the specific resting metabolic rates of major organs and tissues in adults. The following values expressed as in kcal kg^{-1} d^{-1} were indeed calculated: 200 for liver, 240 for brain, 440 for heart and kidneys, 13 for skeletal muscle, 4.5 for adipose tissue, and 12 for the other organs (Wang et al., 2010). Accordingly, the kidney is second only to the heart in terms of mitochondrial abundance (Pagliarini et al., 2008) and oxygen consumption under resting conditions (O'Connor, 2006). This scenario correlates well with

the high request of ATP by the kidney to remove waste from the blood, to reabsorb nutrients, to modulate the balance of electrolytes and fluid, to maintain the acid-base homeostasis, and to regulate the blood pressure (Bhargava and Schnellmann, 2017). While all kidney districts have a high request for ATP, the mechanism of ATP production is cell type-dependent. Podocytes, endothelial and mesangial cells first use glucose to produce ATP for basal cell processes (Forbes, 2016). On the contrary, proximal tubule cells, which constitute 90% of the outer kidney cortex, are the main ATP consuming cells in the kidney and use fuels such as lactate, glutamine, and fatty acids (Forbes and Thorburn, 2018). These cells are completely dependent on the oxidative phosphorylation, i.e., aerobic respiration, as the primary mechanism of ATP production (Bhargava and Schnellmann, 2017). In good agreement with such a metabolic requirement, non-esterified fatty acids, such as palmitate, are among the preferred substrates for ATP production via β-oxidation. Indeed, the efficiency of ATP production from palmitate is higher than that from glucose. Proximal tubules use the majority of the generated ATP to perform active transport and reabsorption of solutes including glucose, amino acids, phosphate, bicarbonate and various filtered low molecular weight proteins, as well as to produce the key urinary buffer ammonium (NH_4^+). Indeed, all the secondary active transport processes depend on the Na^+-K^+-ATPase localized on the basolateral side of the proximal tubular cell (Bhargava and Schnellmann, 2017). Another reason to avoid glycolysis for producing ATP is that proximal tubules harbor a high glucose concentration gradient from their luminal (urinary filtrate) to basal (blood) side for glucose reabsorption. Hence, using glucose to derive energy could be toxic, especially under conditions of metabolic imbalance such as in diabetes (Forbes, 2016). Although β-oxidation of fatty acids is the most efficient mechanism for producing ATP, this pathway implies a high oxygen request; this makes proximal tubules more susceptible than other cell types to changes in oxygen levels. Indeed, a lower oxygen supply can lead to impaired β-oxidation and ATP synthesis which in turn can trigger kidney injury. In this minireview, the network of transporters and enzymes responsible for the mitochondrial fatty acid oxidation will be dealt with focusing on derangements that underlie kidney injury with special reference to the Acute Kidney Disease (AKI).

THE MITOCHONDRIAL FATTY ACID β-OXIDATION AND THE CARNITINE SHUTTLE FOR ATP PRODUCTION

Fatty acid oxidation mainly occurs in mitochondria and involves a repeated sequence of reactions that result in the conversion of fatty acids to acetyl-CoA. Fatty acids are mainly taken up by proximal tubule cells through CD36. This is a fatty acid transporter that plays several roles in human lipid metabolism such as fatty acids storage in adipose tissues and fatty acids supply to produce ATP by β-oxidation in proximal tubule cells (Yokoi and Yanagita, 2016; **Figure 1**). Alternatively, fatty acids result from the deacylation of cellular phospholipids mediated by phospholipase A2 (PLA2). Independently from their origin, fatty acids are then activated in the cytosol by the long-chain acyl-CoA synthetase producing long-chain acyl-CoA. Due to the lack of an acyl-CoA transporter in the mitochondrial inner membrane, the acyl group is transferred to the shuttle molecule carnitine for translocation into the matrix (Indiveri et al., 2011; Casals et al., 2016). The shuttling process starts with the action of CPT1 that catalyzes the conversion of acyl-CoAs into acylcarnitines (**Figure 1**). CPT1 was identified as part of a large protein complex of the mitochondrial outer membrane, protruding toward the cytosol, which includes long-chain acyl-CoA synthetase (ACSL) and the voltage-dependent anion channel (VDAC) also known as porin (Lee et al., 2011). The formed acylcarnitines cross the inner mitochondrial membrane through the action of the carnitine acylcarnitine carrier (CAC – SLC25A20). This transporter mediates an antiport reaction coupling the entry of acylcarnitine into the matrix with the exit of free carnitine (**Figure 1**). It exhibits a higher affinity for acylcarnitines with longer carbon chains and is located in the inner mitochondrial membrane where takes contact with CPT2 located on the matrix side of the inner mitochondrial membrane (Lee et al., 2011). CPT2 transfers back the acyl groups from acylcarnitines to the mitochondrial CoA forming the mitochondrial acyl-CoAs that undergo β-oxidation (**Figure 1**). Each round of β-oxidation requires the sequential action of at least 4 enzymes: the first enzyme is the acyl-CoA dehydrogenase (ACAD) that catalyzes the α,β-dehydrogenation of acyl-CoA. Five types of ACADs are known which are classified according to the substrate specificity; hence, each isoform has a preference for substrates with different chain lengths. These redox enzymes use FAD as a cofactor. The protein expression of medium Acyl-CoA dehydrogenase (MCAD) in the renal tubule is higher than in glomeruli, confirming the important role of FAO in this tissue (Uhlen et al., 2015). The electrons from $FADH_2$ are subsequently transferred to the electron transferring factor (ETF), which in turn releases the electrons to the ETF dehydrogenase coupled with the electron transport chain via the Coenzyme Q. The second step of the β-oxidation is catalyzed by the 2-enoyl-CoA hydratase (ECH), while the third step is catalyzed by 2 distinct forms of the NAD^+ dependent 3-hydroxyacyl-CoA dehydrogenase that show different specificities: the long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) and the short/medium-chain hydroxyacyl-CoA dehydrogenase (HAD). The fourth step is catalyzed by the long-chain 3-ketoacyl-CoA thiolase (HADHB) with a broad chain-length preference. After this first round of reactions, the two carbon shortened Acyl-CoA is recycled through subsequent rounds of β-oxidation reactions generating Acetyl-CoA that enters the TCA cycle allowing the ATP synthesis by the oxidative phosphorylation (**Figure 1**). As above described, the availability of carnitine for fatty acid transport into mitochondria is crucial for the accomplishment of FAO. Due to its fundamental role, it is not surprising that the carnitine homeostasis is strictly regulated (Pochini et al., 2019). The endogenous carnitine synthesis is insufficient to meet the human body need, thus, about half of the carnitine pool is

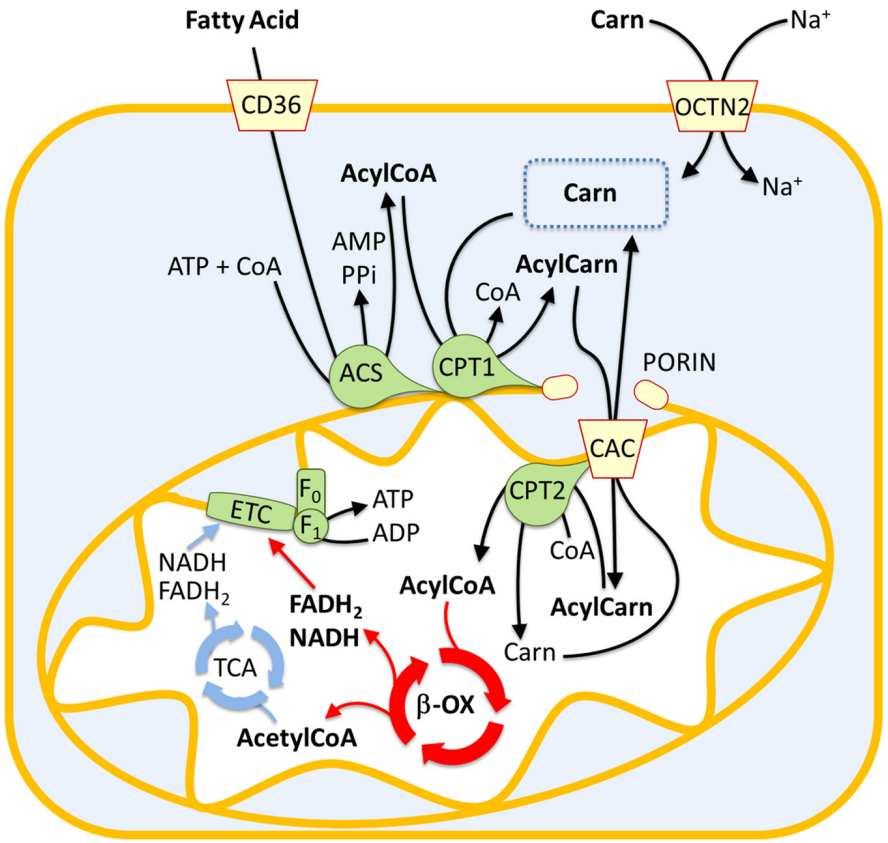


FIGURE 1 | Involvement of the carnitine shuttle in the mitochondrial β-oxidation pathway. The picture represents a sketch of renal proximal tubule cells. Fatty acids (FA) enter the cytosol via CD36. FA are converted in acyl-CoA by acetyl-CoA synthetase (ACS) and then transferred to the mitochondrial matrix by the carnitine shuttle constituted by carnitine-palmitoyltransferase 1 (CPT1), carnitine-acylcarnitine carrier (CAC), and carnitine-palmitoyltransferase 2 (CPT2). Acyl-CoA undergoes β-oxidation (β-ox) with the production of acetyl-CoA that enters the tricarboxylic acid cycle (TCA). NADH and $FADH_2$ generated by β-oxidation and TCA are the electron donors for the electron transport chain (ETC). The Organic Cation Transporter Novel 2 (OCTN2) mediates carnitine entry in the cytosol by a sodium dependent transport reaction.

absorbed from the diet. The plasma membrane Organic Cation Transporter Novel 2 (OCTN2 – SLC22A5), is the major player for carnitine absorption and distribution to the various tissues. The transporter also plays a major role in the renal tubular reabsorption of carnitine which concurs in maintaining the homeostasis (Pochini et al., 2019). Kidney diseases are associated to some extent with carnitine homeostasis derangements. Indeed, some patients with kidney diseases develop carnitine deficiency (Hedayati, 2006).

FATTY ACID OXIDATION IMPAIRMENT AND THE ONSET OF KIDNEY DISEASES

The link between lipid accumulation and kidney disease was suggested for the first time in 1860 by Rudolf Virchow. In a lecture at the Pathological Institute of Berlin, he mentioned the "fatty degeneration of the renal epithelium as a stage of Bright's disease," which is the historical name for glomerulonephritis indicating a range of immune-mediated disorders that cause inflammation of glomeruli and other compartments of the kidney (Chadban and Atkins, 2005). Lipid accumulation reflects an imbalance between fatty acid utilization and fatty acid supply. Since triglycerides buffer fatty acid excess, the overload is often visible as lipid droplets. Several studies suggested that glomeruli and proximal tubules are the most susceptible to lipid accumulation and hence linked to kidney dysfunction (**Figure 2**; Bobulescu, 2010). A typical example is the diabetic nephropathy (DN), a life-threatening pathological condition occurring in the 50% of diabetes mellitus patients with End Stage Renal Disease (ESRD). The molecular features of DN are not yet completely understood; however, lipotoxicity caused by fatty acid deposition and tubule-interstitial fibrosis characterized by epithelial-to-mesenchymal transition (EMT), seem to be hallmarks of DN. Interestingly, a study conducted on human proximal tubular cells, cultured in high glucose medium to mimic diabetic condition, shed light on the events underlying DN onset. It seems that the lipotoxicity occurs before the EMT (Xu et al., 2014). In line with this, the silencing of a key enzyme for fatty acid biosynthesis, namely acetyl-CoA carboxylase 2 (ACC2), increased the β-oxidation rate with a reduction of lipotoxicity and reversion of the EMT morphological changes

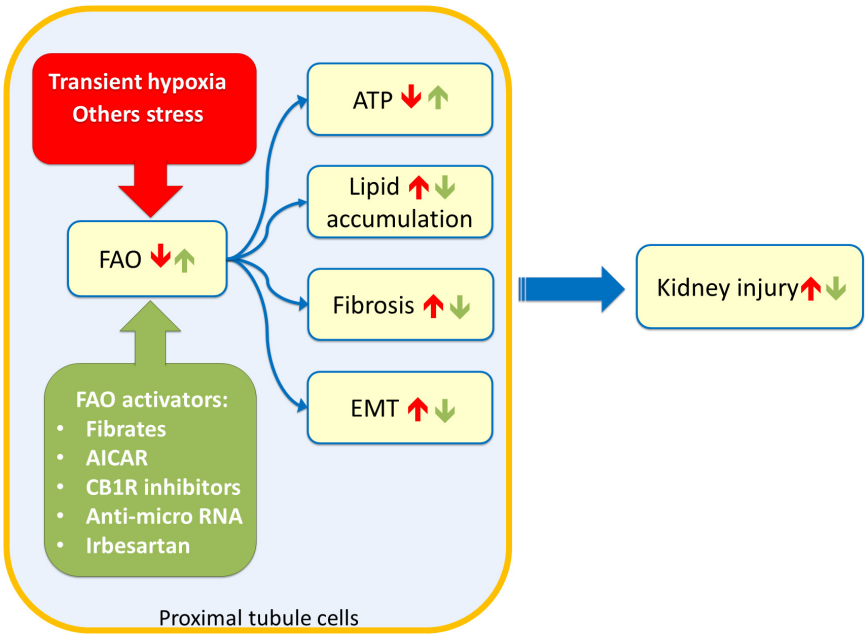


FIGURE 2 | Sketch of kidney tubule cell injury. Stress such as transient hypoxia (red box) can impair Fatty Acid Oxidation (FAO) of proximal tubule cells leading to ATP depletion, lipid accumulation, fibrosis phenotype, and Epithelial to Mesenchimal Transitiona (EMT). These events culminate in chronic as well as acute kidney injury. Stimulation of FAO trough drugs or innovative therapy approaches (green box) can ameliorate the kidney functions after insults. 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR); renal cannabinoid-1 receptor (CB1R).

(Xu et al., 2014). The effect on the β-oxidation rescue is due to reduced production of malonyl-CoA by ACC2 with consequent activation of CPT1, un upstream enzyme of FAO (**Figure 1**). In line with this, another study reported that tubular epithelial cells treated with the CPT1 inhibitor etomoxir showed an increase of gene expression typical of fibrosis, such as α-SMA and Vimentin (Kang et al., 2015); conversely, a model of induced kidney fibrosis treated with the CPT1 activator C75 showed an improvement of the fibrosis phenotype (Kang et al., 2015). Another pathological condition characterized by fatty acid accumulation causing lipotoxicity is one of the most common forms of acute kidney injury (AKI), which is the ischemic renal injury (IRI). The mechanism of lipid accumulation in IRI is not completely understood. Indeed, the accumulation of triglycerides and cholesterol, following the onset of ischemia, seems protective due to the buffer effects against fatty acids. Later, with the progress of the ischemia, the lipid accumulation, visible as droplets, may cause acute kidney injury (Erpicum et al., 2018). During ischemia/reperfusion injury, a decline in the activity of CPT1 has been observed resulting in reduced uptake of fatty acids in the mitochondrial matrix and, hence, reduced FAO. Treating kidney with the CPT1 activator C75, the FAO increased with consequent improvement of renal morphology (Idrovo et al., 2012b). The IRI is characterized by an increase in oxidative stress. This has an influence also on the activity of the central component of the carnitine shuttle, CAC (**Figure 1**). This membrane transporter is located in the inner mitochondrial membrane and possesses six cysteine residues two of which, namely C155 and C136, constitute a redox sensing centre. Indeed, H_2O_2 at toxic concentrations, triggers the formation of a disulfide bridge between C155 and C136 switching off the transport activity. In this respect, it is interesting that the gas transmitters NO or H_2S as well as GSSG act on the same cysteines modulating the CAC activity. This, in turn, may influence the mitochondrial uptake of fatty acids (Giangregorio et al., 2013, 2016; Tonazzi et al., 2013, 2017). In line with this, preconditioning with propionyl-carnitine, to replenish the carnitine pool for FAO, is protective against IRI damage in a rat model (Erpicum et al., 2018). Another event occurring during IRI and, then, characteristic of AKI pathogenesis, is hypoxia (**Figure 2**). In this condition, the NADH: NAD^+ ratio in the mitochondria increases due to the reduction in NADH oxidation rate in the respiratory chain (Simon and Hertig, 2015). Indeed, in the absence of adequate oxygen supply, the electron transport chain is slowed down with the accumulation of NADH which, in turn, inhibits NADH-producing reactions. These phenomena have important impacts on lipid metabolism: for example, the above-mentioned 3-hydroxyacyl-CoA dehydrogenase is one of the rate-controlling enzymes of FAO and requires NAD^+ as a cofactor; this enzyme is impaired when the NADH: NAD^+ ratio increases with the reduction of FAO and lipid accumulation (Simon and Hertig, 2015). In good agreement with the existence of a coordinate network between enzymes and transporters for accomplishing FAO (**Figure 1**), the expression of these proteins is mainly under the control of the same group of transcription factors, namely peroxisome proliferator-activated receptors PPAR-α, PPAR-β/δ, and PPAR-γ. The PPARs are predominantly expressed in metabolically active cells, such as

the renal proximal tubules (Varga et al., 2011). PPARs can recognize a large class of endogenous ligands among which some ω3 and ω6-polyunsaturated fatty acids (PUFAs) and some saturated fatty acids, such as myristic acids. PPARs interact with the members of another subfamily of nuclear receptors, namely RXRs (retinoid X receptor), forming heterodimers. Upon this binding, the heterodimer translocates in the nucleus and interacts with specific DNA responsive elements with consequent increased expression of FAO enzymes as well as of the fatty acid transporter CD36. The plasma membrane transporter OCTN2 and the mitochondrial transporter CAC are also regulated by some of the PPAR family members, among which PPAR-α (D'Argenio et al., 2010; Indiveri et al., 2011; Varga et al., 2011; Qu et al., 2014; Zhou et al., 2014). Interestingly, a link between lipid accumulation, AKI, and PPARs has been demonstrated. Indeed, the renal fibrosis and the epithelial-to-mesenchymal transition, characteristic of AKI are induced by the secretion of the transforming growth factor β1 (TGF-β1) from tubular epithelial cells. TGF-β1 is a cytokine that reduces the expression of PPAR-α and the PPAR-γ coactivator-1a (PPARGC1A) with consequently reduced expression of CPT1 and of the FAO enzyme Peroxisomal acyl-coenzyme A oxidase 1 (Acox1) which catalyzes the desaturation of acyl-CoAs to 2-*trans*-enoyl-CoAs. The effects of TGF-β1 are exerted through the transcription factor SMAD3 which binds to an intronic region of PPAR-α gene. This interaction affects the expression of all the downstream targets causing significant metabolic depression, low cellular ATP content and, then, lipid accumulation (Kang et al., 2015). It has also been demonstrated the microRNA-21, upregulated in different models of renal fibrosis, can silence PPAR-α expression triggering FAO blockade and lipotoxicity. Accordingly, miR-21(−/−) mice suffered less interstitial fibrosis in response to kidney injury (Chau et al., 2012). Therefore, miR-21 and PPAR-α act coordinately in regulating FAO in renal injury. Another microRNA, miR-33, seems to have a role in lipid accumulation and development of fibrosis in the kidney (Price et al., 2019).

THERAPEUTIC INTERVENTIONS FOR RESCUING FATTY ACID OXIDATION

As described above, dysfunctions of the mitochondrial β-oxidation trigger kidney injury, therefore, mitochondria represent a potential drug target. Several novel compounds that promote mitochondrial function are currently under development (Szeto, 2017). A growing number of data suggests that the activation of PPARα can be protective (Cheng et al., 2010). The first class of compounds tested to obtain PPARα activation was that of fibrates (**Figure 2**). In a murine model, it was demonstrated that the treatment with high-dose of PPARα agonists worsened the tubular damage; while, pre-treatment with a low dose of clofibrate prevented acute tubular injuries without accumulation of metabolites harmful for renal function. The tubular protective effects appeared to be associated with the counteraction of PPARα deterioration, resulting in a maintenance of FAO, a decrease of intracellular accumulation of undigested FFAs, and attenuation of disease developmental factors including oxidative stress, apoptosis, and NFκB activation (Takahashi et al., 2011). These effects are common to other fibrates, such as fenofibrate, that significantly attenuated the degree of renal dysfunction and inflammation caused by ischemia/reperfusion (I/R) injury. Interestingly, fenofibrate did not protect PPAR-α(−/−) mice against I/R (Patel et al., 2009). Furthermore, the PPAR- α agonist, WY14643, seems to reduce the renal dysfunction and injury associated with I/R (Sivarajah et al., 2002), while benzafibrate inhibits cisplatin-mediated injury by preventing the proximal tubule cell death (Nagothu et al., 2005). An alternative to fibrates is represented by the angiotensin II receptor blocker irbesartan, which increased PPARα signaling in the liver as well as in the kidney (**Figure 2**). The effect of irbesartan seems to be safe also for the patient with CKD (Harada et al., 2016). Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is another key transcriptional regulator of mitochondrial biogenesis and function (Li and Susztak, 2018). A decrease in PGC-1α expression is commonly observed in mice and patients with acute and chronic kidney disease. Increasing PGC-1α expression in renal tubule cells restores energy deficit and has been shown to protect from acute and chronic kidney disease. Stimulation of the PGC-1α pathway could provide a potential intervention strategy, however, endothelial cells and podocytes are negatively influenced by excessive increased PGC-1α (Li and Susztak, 2018). Another approach to improve renal function after IRI is represented by a treatment combining carnitine and 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR), which activates CPT1 through the adenosine monophosphate-activated protein kinase (AMPK). This combined treatment significantly increased CPT1 activity and ATP levels with lowered renal malondialdehyde, a marker of oxidative stress, and serum TNF-α levels (Idrovo et al., 2012a). An additional strategy for rescuing the ATP level after AKI is the treatment with the mitochondria-targeted compound SS-31; it belongs to the Szeto-Schiller (SS) peptides family, discovered by Hazel H. Szeto and Peter W. Schiller. SS-31 selectively targets and concentrates in the inner mitochondrial membrane where binds with a high affinity the anionic phospholipid cardiolipin (Szeto and Schiller, 2011). Interestingly, cardiolipin plays a structural role in the cristae formation, in the organization of the respiratory supercomplexes and is essential for the transport activity of CAC (Tonazzi et al., 2005). The SS-31/cardiolipin complex inhibited peroxidase activity of cytochrome c, which catalyzes cardiolipin peroxidation and results in mitochondrial damage, FAO decrease and lipid accumulation (Birk et al., 2013; Szeto, 2014, 2017). A novel class of therapeutics is represented by the anti-miR oligonucleotides (**Figure 2**); indeed, several advancements have been made in RNA stability *in vivo*. Interestingly, the latest generation of anti-miR oligonucleotides can be delivered by weekly subcutaneous injection without loss of activity. Recent studies using anti-miR-21 and anti-miR-33 oligonucleotides demonstrated protective renal action (Chau et al., 2012; Price et al., 2019). In particular treatment with anti-miR-33 significantly increases protein levels of CPT1a (the kidney and live isoform) as well as Peroxisomal carnitine

O-octanoyl transferase (CROT) which are involved in FAO, reducing the development of fibrosis (Price et al., 2019). A great deal of attention is currently placed on the effects of obesity on the development of kidney disease. Obesity-induced nephropathy is linked to the activation of the renal cannabinoid-1 receptor (CB1R). Inhibition or selective deletion of CB1R in the renal proximal tubule cells markedly attenuated lipid accumulation, inflammation, and fibrosis in the kidney (**Figure 2**). These effects are associated with enhanced fatty acid β-oxidation and increased activation of the liver kinase B1/AMP-activated protein kinase signaling pathway (Udi et al., 2017).

CONCLUSION

Mitochondrial dysfunction is commonly observed in many nephropathies. Renal cell repair is dependent on the ability of mitochondria to rescue the production of ATP. Thus, restoring mitochondrial β-oxidation might reverse or attenuate renal failure. PPAR-α agonists prevent tubule cell death and intracellular lipid accumulation. In this scenario, a deeper understanding of the tissue specificity effects and the molecular mechanisms of PPAR-α is mandatory to develop novel and safer therapies. The most innovative pharmacological approach is the use of anti-micro RNA oligonucleotides. This new strategy can now be suitable thanks to the advancement in the stability of these molecules. A weekly subcutaneous injection is sufficient to deliver the anti-miR without loss of activity.

AUTHOR CONTRIBUTIONS

LC contributed to collecting bibliography, writing the manuscript, and conceiving and creating the figures. MS contributed to writing the manuscript and creating the figures. NG, AT, and MB were involved in the critical revision and writing of the manuscript. CI supervised the work and wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by PRIN (Progetti di Ricerca di Rilevante Interesse Nazionale) Project No. 2017PAB8EM to CI granted by MIUR (Ministry of Education, University and Research) Italy.

REFERENCES

Bhargava, P., and Schnellmann, R. G. (2017). Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.* 13, 629–646. doi: 10.1038/nrneph.2017.107

Birk, A. V., Liu, S., Soong, Y., Mills, W., Singh, P., Warren, J. D., et al. (2013). The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. *J. Am. Soc. Nephrol.* 24, 1250–1261. doi: 10.1681/asn.2012121216

Bobulescu, I. A. (2010). Renal lipid metabolism and lipotoxicity. *Curr. Opin. Nephrol. Hypertens.* 19, 393–402. doi: 10.1097/mnh.0b013e32833aa4ac

Casals, N., Zammit, V., Herrero, L., Fadó, R., Rodríguez-Rodríguez, R., and Serra, D. (2016). Carnitine palmitoyltransferase 1C: from cognition to cancer. *Prog. Lipid Res.* 61, 134–148. doi: 10.1016/j.plipres.2015.11.004

Chadban, S. J., and Atkins, R. C. (2005). Glomerulonephritis. *Lancet* 365, 1797–1806.

Chau, B. N., Xin, C., Hartner, J., Ren, S., Castano, A. P., Linn, G., et al. (2012). MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Sci. Transl. Med.* 4:121ra18. doi: 10.1126/scitranslmed.3003205

Cheng, C. F., Chen, H. H., and Lin, H. (2010). Role of PPARalpha and its agonist in renal diseases. *PPAR Res.* 2010:345098.

D'Argenio, G., Petillo, O., Margarucci, S., Torpedine, A., Calarco, A., Koverech, A., et al. (2010). Colon OCTN2 gene expression is up-regulated by peroxisome proliferator-activated receptor gamma in humans and mice and contributes to local and systemic carnitine homeostasis. *J. Biol. Chem.* 285, 27078–27087. doi: 10.1074/jbc.m110.109678

Elia, M. (1992). Organ and tissue contribution to metabolic rate. Energy metabolism, tissue determinants and cellular corollaries. *Am. J. Clin. Nutr.* 92, 61–80.

Erpicum, P., Rowart, P., Defraigne, J. O., Krzesinski, J. M., and Jouret, F. (2018). What we need to know about lipid-associated injury in case of renal ischemia-reperfusion. *Am. J. Physiol. Renal Physiol.* 315, F1714–F1719.

Forbes, J. M. (2016). Mitochondria-power players in kidney function? *Trends Endocrinol. Metab.* 27, 441–442. doi: 10.1016/j.tem.2016.05.002

Forbes, J. M., and Thorburn, D. R. (2018). Mitochondrial dysfunction in diabetic kidney disease. *Nat. Rev. Nephrol.* 14, 291–312. doi: 10.1038/nrneph.2018.9

Giangregorio, N., Palmieri, F., and Indiveri, C. (2013). Glutathione controls the redox state of the mitochondrial carnitine/acylcarnitine carrier Cys residues by glutathionylation. *Biochim. Biophys. Acta* 1830, 5299–5304. doi: 10.1016/j.bbagen.2013.08.003

Giangregorio, N., Tonazzi, A., Console, L., Lorusso, I., De Palma, A., and Indiveri, C. (2016). The mitochondrial carnitine/acylcarnitine carrier is regulated by hydrogen sulfide via interaction with C136 and C155. *Biochim. Biophys. Acta* 1860(1 Pt A), 20–27. doi: 10.1016/j.bbagen.2015.10.005

Harada, M., Kamijo, Y., Nakajima, T., Hashimoto, K., Yamada, Y., Shimojo, H., et al. (2016). Peroxisome proliferator-activated receptor alpha-dependent renoprotection of murine kidney by irbesartan. *Clin. Sci.* 130, 1969–1981. doi: 10.1042/cs20160343

Hedayati, S. S. (2006). Dialysis-related carnitine disorder. *Semin. Dial.* 19, 323–328. doi: 10.1111/j.1525-139x.2006.00180.x

Idrovo, J. P., Yang, W. L., Matsuda, A., Nicastro, J., Coppa, G. F., and Wang, P. (2012a). Post-treatment with the combination of 5-aminoimidazole-4-carboxyamide ribonucleoside and carnitine improves renal function after ischemia/reperfusion injury. *Shock* 37, 39–46. doi: 10.1097/shk.0b013e31823185d7

Idrovo, J. P., Yang, W. L., Nicastro, J., Coppa, G. F., and Wang, P. (2012b). Stimulation of carnitine palmitoyltransferase 1 improves renal function and attenuates tissue damage after ischemia/reperfusion. *J. Surg. Res.* 177, 157–164. doi: 10.1016/j.jss.2012.05.053

Indiveri, C., Iacobazzi, V., Tonazzi, A., Giangregorio, N., Infantino, V., Convertini, P., et al. (2011). The mitochondrial carnitine/acylcarnitine carrier: function, structure and physiopathology. *Mol. Aspects Med.* 32, 223–233. doi: 10.1016/j.mam.2011.10.008

Kang, H. M., Ahn, S. H., Choi, P., Ko, Y. A., Han, S. H., Chinga, F., et al. (2015). Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat. Med.* 21, 37–46. doi: 10.1038/nm.3762

Lee, K., Kerner, J., and Hoppel, C. L. (2011). Mitochondrial carnitine palmitoyltransferase 1a (CPT1a) is part of an outer membrane fatty acid transfer complex. *J. Biol. Chem.* 286, 25655–25662. doi: 10.1074/jbc.m111.228692

Li, S. Y., and Susztak, K. (2018). The Role of peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) in Kidney disease. *Semin. Nephrol.* 38, 121–126. doi: 10.1016/j.semnephrol.2018.01.003

Nagothu, K. K., Bhatt, R., Kaushal, G. P., and Portilla, D. (2005). Fibrate prevents cisplatin-induced proximal tubule cell death. *Kidney Int.* 68, 2680–2693. doi: 10.1111/j.1523-1755.2005.00739.x

O'Connor, P. M. (2006). Renal oxygen delivery: matching delivery to metabolic demand. *Clin. Exp. Pharmacol. Physiol.* 33, 961–967. doi: 10.1111/j.1440-1681.2006.04475.x

Pagliarini, D. J., Calvo, S. E., Chang, B., Sheth, S. A., Vafai, S. B., Ong, S. E., et al. (2008). A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 134, 112–123. doi: 10.1016/j.cell.2008.06.016

Patel, N. S., di Paola, R., Mazzon, E., Britti, D., Thiemermann, C., and Cuzzocrea, S. (2009). Peroxisome proliferator-activated receptor-alpha contributes to the resolution of inflammation after renal ischemia/reperfusion injury. *J. Pharmacol. Exp. Ther.* 328, 635–643. doi: 10.1124/jpet.108.146191

Pochini, L., Galluccio, M., Scalise, M., Console, L., and Indiveri, C. (2019). OCTN: a small transporter subfamily with great relevance to human pathophysiology, drug discovery, and diagnostics. *SLAS Discov.* 24, 89–110. doi: 10.1177/2472555218812821

Price, N. L., Miguel, V., Ding, W., Singh, A. K., Malik, S., Rotllan, N., et al. (2019). Genetic deficiency or pharmacological inhibition of miR-33 protects from kidney fibrosis. *JCI Insight.* 4:e131102.

Qu, Q., Qu, J., Guo, Y., Zhou, B. T., and Zhou, H. H. (2014). Luteolin potentiates the sensitivity of colorectal cancer cell lines to oxaliplatin through the PPARgamma/OCTN2 pathway. *Anticancer Drugs* 25, 1016–1027. doi: 10.1097/cad.0000000000000125

Simon, N., and Hertig, A. (2015). Alteration of fatty acid oxidation in tubular epithelial cells: from acute kidney injury to renal fibrogenesis. *Front. Med.* 2:52. doi: 10.3389/fmed.2015.00052

Sivarajah, A., Chatterjee, P. K., Hattori, Y., Brown, P. A., Stewart, K. N., Todorovic, Z., et al. (2002). Agonists of peroxisome-proliferator activated receptor-alpha (clofibrate and WY14643) reduce renal ischemia/reperfusion injury in the rat. *Med. Sci. Monit.* 8, BR532–BR539.

Szeto, H. H. (2014). First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br. J. Pharmacol.* 171, 2029–2050. doi: 10.1111/bph.12461

Szeto, H. H. (2017). Pharmacologic approaches to improve mitochondrial function in AKI and CKD. *J. Am. Soc. Nephrol.* 28, 2856–2865. doi: 10.1681/asn.2017030247

Szeto, H. H., and Schiller, P. W. (2011). Novel therapies targeting inner mitochondrial membrane–from discovery to clinical development. *Pharm. Res.* 28, 2669–2679. doi: 10.1007/s11095-011-0476-8

Takahashi, K., Kamijo, Y., Hora, K., Hashimoto, K., Higuchi, M., Nakajima, T., et al. (2011). Pretreatment by low-dose fibrates protects against acute free fatty acid-induced renal tubule toxicity by counteracting PPARalpha deterioration. *Toxicol. Appl. Pharmacol.* 252, 237–249. doi: 10.1016/j.taap.2011.02.012

Tonazzi, A., Console, L., and Indiveri, C. (2013). Inhibition of mitochondrial carnitine/acylcarnitine transporter by H(2)O(2): molecular mechanism and possible implication in pathophysiology. *Chem. Biol. Interact.* 203, 423–429. doi: 10.1016/j.cbi.2013.01.006

Tonazzi, A., Giangregorio, N., Console, L., De Palma, A., and Indiveri, C. (2017). Nitric oxide inhibits the mitochondrial carnitine/acylcarnitine carrier through reversible S-nitrosylation of cysteine 136. *Biochim. Biophys. Acta Bioenerg.* 1858, 475–482. doi: 10.1016/j.bbabio.2017.04.002

Tonazzi, A., Giangregorio, N., Indiveri, C., and Palmieri, F. (2005). Identification by site-directed mutagenesis and chemical modification of three vicinal cysteine residues in rat mitochondrial carnitine/acylcarnitine transporter. *J. Biol. Chem.* 280, 19607–19612. doi: 10.1074/jbc.m411181200

Udi, S., Hinden, L., Earley, B., Drori, A., Reuveni, N., Hadar, R., et al. (2017). Proximal tubular cannabinoid-1 receptor regulates obesity-induced CKD. *J. Am. Soc. Nephrol.* 28, 3518–3532. doi: 10.1681/asn.2016101085

Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., et al. (2015). Proteomics. Tissue-based map of the human proteome. *Science* 347:1260419.

Varga, T., Czimmerer, Z., and Nagy, L. (2011). PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. Biophys. Acta* 1812, 1007–1022. doi: 10.1016/j.bbadis.2011.02.014

Wang, Z., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., et al. (2010). Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure. *Am. J. Clin. Nutr.* 92, 1369–1377. doi: 10.3945/ajcn.2010.29885

Xu, Y., Huang, J., Xin, W., Chen, L., Zhao, X., Lv, Z., et al. (2014). Lipid accumulation is ahead of epithelial-to-mesenchymal transition and therapeutic intervention by acetyl-CoA carboxylase 2 silence in diabetic nephropathy. *Metabolism* 63, 716–726. doi: 10.1016/j.metabol.2014.02.010

Yokoi, H., and Yanagita, M. (2016). Targeting the fatty acid transport protein CD36, a class B scavenger receptor, in the treatment of renal disease. *Kidney Int.* 89, 740–742. doi: 10.1016/j.kint.2016.01.009

Zhou, X., Ringseis, R., Wen, G., and Eder, K. (2014). Carnitine transporter OCTN2 and carnitine uptake in bovine kidney cells is regulated by peroxisome proliferator-activated receptor beta/delta. *Acta Vet. Scand.* 56:21. doi: 10.1186/1751-0147-56-21

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 14 July 2020
doi: 10.3389/fphys.2020.00755

Manganese Superoxide Dismutase Dysfunction and the Pathogenesis of Kidney Disease

Munehiro Kitada[1,2], Jing Xu[1], Yoshio Ogura[1], Itaru Monno[1] and Daisuke Koya[1,2]*

[1] Department of Diabetology and Endocrinology, Kanazawa Medical University, Uchinada, Japan, [2] Division of Anticipatory Molecular Food Science and Technology, Medical Research Institute, Kanazawa Medical University, Uchinada, Japan

The mitochondria are a major source of reactive oxygen species (ROS). Superoxide anion ($O_2^{\bullet-}$) is produced by the process of oxidative phosphorylation associated with glucose, amino acid, and fatty acid metabolism, resulting in the production of adenosine triphosphate (ATP) in the mitochondria. Excess production of reactive oxidants in the mitochondria, including $O_2^{\bullet-}$, and its by-product, peroxynitrite ($ONOO^-$), which is generated by a reaction between $O_2^{\bullet-}$ with nitric oxide ($NO^{\bullet}$), alters cellular function via oxidative modification of proteins, lipids, and nucleic acids. Mitochondria maintain an antioxidant enzyme system that eliminates excess ROS; manganese superoxide dismutase (Mn-SOD) is one of the major components of this system, as it catalyzes the first step involved in scavenging ROS. Reduced expression and/or the activity of Mn-SOD results in diminished mitochondrial antioxidant capacity; this can impair the overall health of the cell by altering mitochondrial function and may lead to the development and progression of kidney disease. Targeted therapeutic agents may protect mitochondrial proteins, including Mn-SOD against oxidative stress-induced dysfunction, and this may consequently lead to the protection of renal function. Here, we describe the biological function and regulation of Mn-SOD and review the significance of mitochondrial oxidative stress concerning the pathogenesis of kidney diseases, including chronic kidney disease (CKD) and acute kidney injury (AKI), with a focus on Mn-SOD dysfunction.

Keywords: manganese superoxide dismutase, acute kidney injury, chronic kidney disease, mitochondria, peroxynitrite, posttranslational modification

OPEN ACCESS

Edited by:
Egor Plotnikov, Lomonosov Moscow State University, Russia

Reviewed by:
Adam J. Case, University of Nebraska Medical Center, United States
Yunfeng Zhao, Louisiana State University Health Sciences Center New Orleans, United States

****Correspondence:***
Munehiro Kitada
kitta@kanazawa-med.ac.jp

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *15 May 2020*
Accepted: *11 June 2020*
Published: *14 July 2020*

Citation:
Kitada M, Xu J, Ogura Y, Monno I and Koya D (2020) Manganese Superoxide Dismutase Dysfunction and the Pathogenesis of Kidney Disease. Front. Physiol. 11:755. doi: 10.3389/fphys.2020.00755

INTRODUCTION

The prevalence of chronic kidney disease (CKD) has been increasing worldwide (Eckardt et al., 2013). CKD is characterized by the gradual loss of renal function over a period of months to years, ultimately leading to end-stage renal disease (ESRD). CKD has also been recognized as an independent risk factor for cardiovascular disease (CVD) (Go et al., 2004; Gansevoort et al., 2013). In contrast, acute kidney injury (AKI), also known as acute renal failure, is defined as the sudden and rapid loss of renal function, often within 48 h (KDIGO, 2012). AKI is closely associated with the increased risk of developing CKD, and progression is directly dependent on the severity of AKI (Fiorentino et al., 2018). Therefore, to suppress these outcomes, it will be necessary to have a

better understanding of the underlying fundamental mechanisms related to progression and onset of both CKD and AKI.

Reactive oxygen species (ROS) can damage cellular proteins, lipids, and DNA, ultimately leading to cellular dysfunction (Wang et al., 2018). Mitochondria are the major source of endogenous ROS, which depends directly on the metabolic and redox state of the mitochondria. Metabolic state refers to the efficiency of electron transfer from nutrients to molecular oxygen (O_2). Electrons that are leaked from the respiratory chain react with O_2 to produce superoxide anions ($O_2^{\bullet-}$). Mitochondria have ROS scavenging systems via which $O_2^{\bullet-}$ is converted to H_2O_2 by the actions of superoxide dismutase (SOD), including Cu/Zn-SOD and manganese superoxide dismutase (Mn-SOD) (Mailloux, 2015; Kim et al., 2017; Wang et al., 2018). Further decomposition of H_2O_2 to H_2O and O_2 is catalyzed by other antioxidative enzymes within the mitochondria, including glutathione peroxidase (GPx) and peroxiredoxin (PRx)/thioredoxin (TRx) (Mailloux, 2015; Kim et al., 2017; Wang et al., 2018). At low levels, ROS are second messengers for signal transduction and may serve to regulate cellular adaptation against several stressors. However, oxidative stress results when ROS production exceeds homeostatic levels due to an imbalance between ROS production and decomposition.

The kidney is a highly metabolic organ with high levels of oxidation within cellular mitochondria. Renal tissues have a large energy demand, most notably within the proximal tubular cells; this is related to their role in reabsorbing critical nutrients after glomerular filtration. As such, the kidney is particularly vulnerable to damage caused by oxidative stress. Previous reports have shown that mitochondrial oxidative stress may be directly linked to mechanisms underlying the onset and progression of both CKD and AKI (Che et al., 2014; He et al., 2017). Excess mitochondrial ROS may promote mitochondrial dysfunction, ATP depletion, inflammation, and apoptosis in association with kidney disease (Che et al., 2014; He et al., 2017). Among ROS scavenging enzymes in the mitochondrial matrix, Mn-SOD has a primary responsibility for $O_2^{\bullet-}$ scavenging within mitochondria; as such, dysfunction of Mn-SOD results in mitochondrial oxidative stress. Collective experimental evidence has suggested a relationship between Mn-SOD dysfunction and the pathogenesis of kidney disease. In this review, we consider the significance of mitochondrial oxidative stress on the pathogenesis of kidney disease, including CKD and AKI, with a focus on the role of Mn-SOD dysfunction.

BIOLOGICAL FUNCTION OF MANGANESE SUPEROXIDE DISMUTASE ON REACTIONS AND TRANSFORMATIONS OF SUPEROXIDE ANION

Human Mn-SOD, encoded by the *sod2* gene, is located on chromosome 6q25.3 (Creagan et al., 1973) (**Figure 1**). The sequence of Mn-SOD is highly conserved, with over 40% homology among proteins from human, yeast, and *Escherichia coli* (Barra et al., 1984). Mn-SOD is a tetrameric enzyme with four identical subunits, each harboring a manganese ion (Mn^{2+}/Mn^{3+}) as a cofactor. Mn-SOD is located primarily within the mitochondrial matrix (Borgstahl et al., 1992; Karnati et al., 2013). By contrast, Cu/Zn-SOD is localized to the mitochondrial inner membrane space.

$O_2^{\bullet-}$ production in mitochondria is closely linked to mitochondrial production of adenosine triphosphate (ATP), which takes place via electron transfer linked to nutrient (glucose, amino acids, and fatty acids) metabolism. Electrons are by-products of the tricarboxylic acid (TCA) cycle enzymes and respiratory complexes that promote a univalent reduction of oxygen (O_2) to $O_2^{\bullet-}$. Superoxide anion ($O_2^{\bullet-}$) is an important signaling molecule but one that can be toxic at high concentrations. SOD enzymes, including Mn-SOD and Cu/Zn-SOD, catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2 in the mitochondrial matrix and the intermembrane space, respectively (Kawamata and Manfredi, 2010; Karnati et al., 2013; Mailloux, 2015). The GPx and PRx/TRx systems convert H_2O_2 to H_2O within the mitochondrial matrix. Alternatively, H_2O_2 that has diffused into the cytoplasm is metabolized by catalase, GPx, and PRx/TRx. $O_2^{\bullet-}$ can also react with nitric oxide ($NO^{\bullet}$) to produce peroxynitrite ($ONOO^-$). The decomposition of $ONOO^-$ gives rise to highly oxidizing intermediates, including nitrogen dioxide ($NO_2^{\bullet}$), and hydroxyl radical ($OH^{\bullet}$); eventually, stable nitrite (NO_3^-) is produced (Tharmalingam et al., 2017; Radi, 2018). As such, elevated levels of $O_2^{\bullet-}$ may result in decreased bioavailability of NO and an increased toxic $ONOO^-$ production. Additionally, $O_2^{\bullet-}$ may reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in iron–sulfur centers in critical proteins, which may lead to enzyme inactivation and concomitant loss of Fe^{2+} from the enzymes, thereby promoting production of H_2O_2 (Imlay, 2003). H_2O_2 may then react with Fe^{2+} to produce hydroxy radicals ($OH^{\bullet}$) via the Fenton/Haber–Weiss reaction. Moreover, protonation of $O_2^{\bullet-}$ may promote formation of the reactive hydroperoxyl radical HO_2.

Oxidative stress induced by the imbalance between ROS production and the scavenging capacity of antioxidant protection mechanisms in the mitochondria leads to the inactivation of endogenous antioxidant systems, impairment of the electron transport, uncoupling of oxidative phosphorylation, and altered membrane permeability. As such, internal oxidative stress-mediated damage may be the main cause of mitochondrial dysfunction. Mn-SOD is the primary antioxidative enzyme that is responsible for scavenging $O_2^{\bullet-}$ in the mitochondrial matrix. Therefore, Mn-SOD dysfunction may result in overproduction of highly reactive oxidants, such as $ONOO^-$ and OH, which may result in mitochondrial dysfunction and disease development.

TRANSCRIPTIONAL REGULATION OF MANGANESE SUPEROXIDE DISMUTASE

The *sod2* gene has three major regulatory regions, namely, a proximal promoter, a 5′ upstream enhancer region, and an intronic enhancer region, which promotes activation or repression of *sod2* gene expression (Xu et al., 2002).

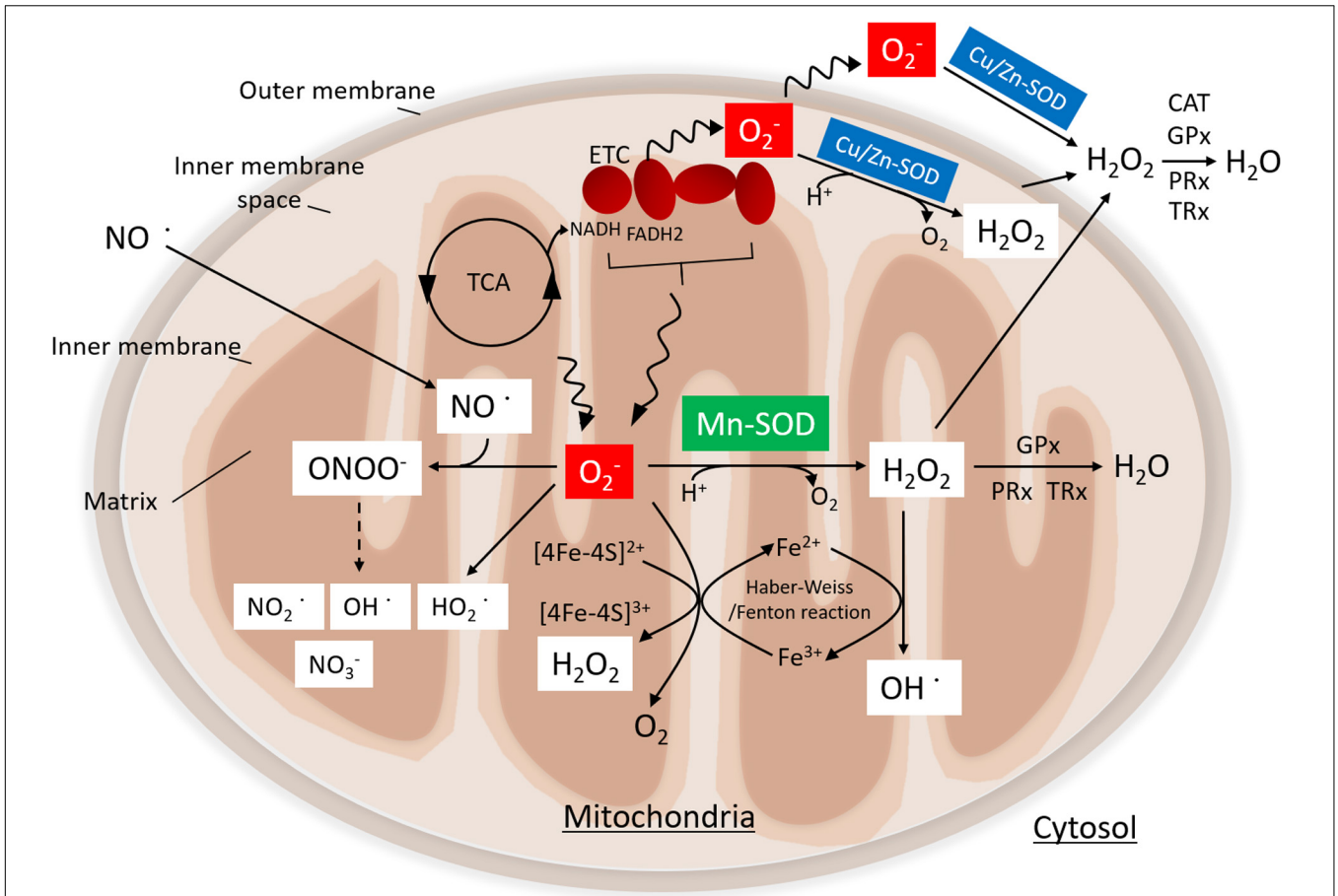


FIGURE 1 | Function of manganese superoxide dismutase (Mn-SOD). Superoxide ($O_2^{\bullet-}$) is produced by the electron transport chain (ETC) during nutrient metabolism by the tricarboxylic acid (TCA) cycle. Mn-SOD is localized in the mitochondrial matrix, where it catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2. By contrast, Cu/Zn-SOD, which is located in the inner membrane space of mitochondria, catalyzes the conversion of $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2). H_2O_2 is further metabolized by the glutathione peroxidase (GPx) and the peroxiredoxin (PRx)/thioredoxin (TRx) system in mitochondrial matrix or by catalase (CAT) GPx and PRx/TRx in the cytosol. $O_2^{\bullet-}$ and nitric oxide (NO•) can react to form peroxynitrite ($ONOO^-$), giving rise to nitrogen dioxide (NO_2•) and hydroxyl radical (OH•); eventually, stable nitrite (NO_3^-) is produced. H_2O_2 is converted to OH via the Fenton/Haber–Weiss reaction. $O_2^{\bullet-}$ can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in iron–sulfur centers of proteins, ultimately resulting in the production of H_2O_2. Moreover, the protonation of $O_2^{\bullet-}$ generates the hydroperoxyl radical, HO_2.

Proximal Promotor of the *sod2* Gene

The basic proximal promoter of the *sod2* gene has no classical TATA or CAAT box, although it is enriched in CG repeats that contain binding sites for specificity protein 1 (Sp1) and activator protein 2 (AP-2) (Zhu et al., 2001; Xu et al., 2002). Sp1 binding to the promoter activates *sod2* gene transcription, whereas AP-2 suppresses the transcriptional activity via competition with Sp1 at its binding site or by limiting its bioavailability via AP-2/Sp1 complex formation. As such, Sp1 and AP-2 regulate the basal level of *sod2* gene transcription via antagonistic mechanisms at the proximal promoter region.

5′ Upstream Enhancer Region of the *sod2* Gene

The 5′ upstream enhancer region of the *sod2* gene includes binding sites for activating protein 1 (AP-1) (Borrello and Demple, 1997), cyclic AMP response element-binding protein (CREB) (Kim et al., 1999), nuclear factor, erythroid 2-like 2 (Nrf2) (Taylor et al., 2008), forkhead box O3 (FoxO3a) (Kops et al., 2002), nuclear factor-kappa B (NF-κB) (Warner et al., 1996), and hypoxia-inducible factor 1α (HIF-1α) (Gao et al., 2013). Binding of transcription factors at these sites within the *sod2* 5′ enhancer leads to an interaction with the basal transcriptional machinery of the proximal promoter, including Sp1. Nrf2 binds to the antioxidant responsive element (ARE) and acts as a master regulator of ARE-responsive antioxidant genes, including *sod2* at homeostasis and in response to induction (Taylor et al., 2008; Li et al., 2015). FoxO-binding elements were identified upstream of the *sod2* transcriptional start site; however, only the site at position −1,249 mediates FoxO3a-dependent transcription of *sod2* gene in quiescent cells (Kops et al., 2002). In pathological conditions, activation of Akt (protein kinase B) promotes nuclear exclusion of FoxO3a via phosphorylation, thereby downregulating the transcription of *sod2* (Kato et al., 2006; Li et al., 2006). FoxO3 is deacetylated

by sirtuins, including SIRT1, SIRT2, and SIRT3, resulting in enhanced DNA binding and *sod2* gene transcription (Jacobs et al., 2008). HIF-1α represses *sod2* gene transcription in association with renal clear cell carcinoma (Gao et al., 2013). High levels of mitochondrial $O_2^{\bullet-}$ that result from the repression of *sod2* gene expression lead to HIF-1α stabilization. Moreover, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) also displays antioxidant defense responses via its role in promoting upregulated expression of antioxidative enzymes, including *sod2* (Valle et al., 2005; St-Pierre et al., 2006; Lu et al., 2010). PGC-1α also plays an important role in mitochondrial biogenesis and respiration (Wu et al., 1999; Lin et al., 2005). Ding et al. (2017) reported that knockdown of SIRT3 inhibited osteogenic differentiation via its role in decreasing the stability of PGC-1α by increasing its acetylation and via downregulated expression of Mn-SOD; this results in elevated levels of ROS and reduced mitochondrial biogenesis and function. Cherry et al. (2014) reported that PGC-1α promotes mitochondrial biogenesis and induces expression of antioxidative enzyme, most notably Nrf2-mediated Mn-SOD expression in the liver of mice of *Staphylococcus aureus*-induced peritonitis.

Binding sites for p53 were identified in the 5′ upstream region of the *sod2* gene (Pani et al., 2000; Drane et al., 2001). p53 exerts its inhibitory action via the prevention of Sp1 binding to the proximal promoter of *sod2* gene (Dhar et al., 2006). Additionally, high levels of p53 expression serve to suppress *sod2* expression, whereas low levels of p53 expression promote NF-κB binding to the enhancer region of the *sod2* gene and increasing *sod2* transcription (Dhar et al., 2010). In contrast, Hussain et al. (2004) reported that the promoter of *sod2* contains p53 consensus binding sites, which located at −2,009 to −2,032 bp upstream of the transcription start site and induction of p53 leads to increased promoter activity and gene expression.

Intronic Enhancer Region of the *sod2* Gene

The intronic enhancer contains a primary binding site for NF-κB (Jones et al., 1997; Xu et al., 1999). NF-κB positively regulates transcription via binding to the intronic enhancer region, which is located in the second intron of the *sod2* gene by promoting interactions with CCAAT/enhancer-binding protein beta (C/EBP-β; Xu et al., 1999; Jones et al., 1997). NF-κB binding at the intronic enhancer region also enhances transcriptional activity of Sp1 in the proximal promoter of *sod2* (Ennen et al., 2011).

POSTTRANSLATIONAL REGULATION OF MANGANESE SUPEROXIDE DISMUTASE ACTIVITY

In addition to transcriptional regulation, previous reports indicated that Mn-SOD function is regulated by posttranslational modifications, including nitration, acetylation, phosphorylation, and glutathionylation.

Nitration

NO, a ubiquitous intracellular messenger, plays crucial roles in a variety of biological processes (Beckman and Koppenol, 1996). Overproduction of NO and $O_2^{\bullet-}$ leads to the formation of the extremely reactive $ONOO^-$, which can result in lipid peroxidation, DNA damage, and protein nitration. $ONOO^-$ can generate covalent modifications of tyrosine (Tyr) residues in proteins, thereby reducing their functionality (**Figure 2**).

$ONOO^-$ leads to Mn-SOD deactivation via nitration of the critical active-site Tyr34 residue (MacMillan-Crow et al., 1998; Yamakura et al., 1998; Yamakura and Kawasaki, 2010) (**Figure 2**). Mn-SOD protects cells by $O_2^{\bullet-}$ scavenging, thereby preventing the interaction of $O_2^{\bullet-}$ with NO. However, Mn-SOD deactivation leads to the amplification of oxidative stress via accumulation of more $O_2^{\bullet-}$ that promotes not just only $ONOO^-$ formation in the mitochondria but also both the onset and progression of several diseases.

Acetylation

Nε-lysine acetylation is recognized as a critical posttranslational modification that regulates protein function, most notably that of histones (Narita et al., 2019). Non-histone protein acetylation modulates numerous cellular processes, including gene transcription, DNA damage and repair, cell division, signal transduction, protein folding, autophagy, and metabolism.

Sirtuins (SIRTs), which are NAD-dependent protein deacetylases, play significant roles in the regulation of protein

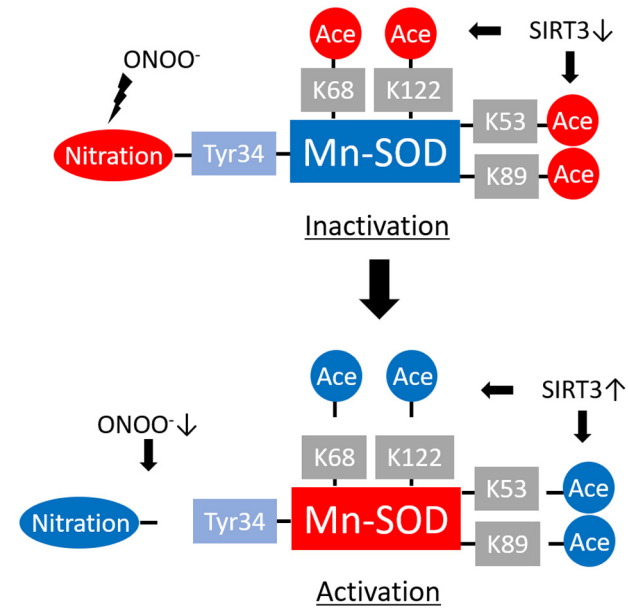


FIGURE 2 | Regulation of manganese superoxide dismutase (Mn-SOD) enzyme activity by translational modifications. $ONOO^-$ promotes Mn-SOD inactivation by nitrating the critical Tyr34 at the enzyme active site. Acetylation of Mn-SOD at K68, K122, K53, and K89 residues also induces Mn-SOD inactivation. Reduced levels of $ONOO^-$ and activation of NAD-dependent deacetylase sirtuin-3 (SIRT3) lead to Mn-SOD reactivation through denitrification or deacetylation of the target residues.

acetylation (Guarente, 2011). SIRT3, SIRT4, and SIRT5 have been identified in the mitochondria (Guarente, 2011; Kitada et al., 2019). The deacetylase activity of SIRT3 is substantially higher than that of SIRT4 or SIRT5; as such, SIRT3 plays a critical role in mitochondrial biology and pathophysiological processes, including redox status, via its capacity to regulate acetylation of lysine (K) residues in mitochondrial proteins (Guarente, 2011; van de Ven et al., 2017; Kitada et al., 2019). Mn-SOD is among the target proteins of SIRT3. Chen et al. (2011) found that Mn-SOD K68 is an important site for SIRT3 regulation in human cells, whereas Tao et al. (2010) identified K122 and Qiu et al. (2010) identified K53 and K89 in Mn-SOD as the main sites of Sirt3 deacetylation in mouse cells (**Figure 2**). Overall, we conclude that SIRT3 deacetylates Mn-SOD at several lysine residues. SIRT3-mediated deacetylation results in enhanced Mn-SOD activity within a given nutrient and redox state and under various pathophysiological conditions. This results in decreased mitochondrial oxidative stress and prevents the progression of cellular aging and aging-related disease.

Other Modifications

Mn-SOD can also be phosphorylated, and this process has a direct impact on enzyme function. Candas et al. (2013) reported that the CyclinB1/CDK1-induced phosphorylation of Mn-SOD at Ser106 led to Mn-SOD activation and increased stability, which was associated with improved mitochondrial function and cellular resistance to apoptosis due to radiation. Additionally, cyclin-dependent kinase 4 (CDK4) located in mitochondria directly phosphorylates Mn-SOD at Ser106, resulting in increased Mn-SOD activity and mitochondrial respiration, notably in skin tissues of mice receiving whole-body low-dose ionizing radiation (Jin et al., 2015). The phosphorylation at Ser106 of Mn-SOD enhances its tetrameric conformation, stability, and enzymatic activity. By contrast, Hopper et al. (2006) reported an inverse correlation between phosphorylation and activity of Mn-SOD in mitochondria isolated from pig heart, and Ca^{2+}-induced dephosphorylation increased Mn-SOD enzymatic activity. Thus, further studies are necessary to clarify how the phosphorylation or dephosphorylation of Mn-SOD is involved in the regulation of Mn-SOD activity.

Additionally, Zhao et al. (2017) identified Mn-SOD oxidation at histidine (His54 and His55), tyrosine (Tyr58), tryptophan (Trp147, Trp149, Trp205, and Trp210), and asparagine (Asn206 and Asn209) residues in human kidney tissues through mass spectrometry. In renal carcinoma cells, Mn-SOD oxidation at histidine (His54 and His55), tyrosine (Tyr58), and tryptophan (Trp147 and Trp149) residues was enhanced and associated with Mn-SOD deactivation (Zhao et al., 2017). John et al. (2009) also found that oxidation at His54 and His55 was associated with Mn-SOD deactivation in human medulloblastoma cells (John et al., 2009).

S-glutathionylation is the term that describes the addition of glutathione to cysteine residues of proteins; this process prevents irreversible oxidation of protein thiols and regulates a diverse array of cellular processes (Mailloux and Willmore, 2014). Mn-SOD can undergo S-glutathionylation within the mitochondria. Hopper et al. (2006) reported S-glutathionylation of recombinant rat Mn-SOD that was generated in *E. coli*. Mn-SOD cysteine (Cys) 196 was identified as a target of S-glutathionylation in the rat (Castellano et al., 2009). Patil et al. (2013) demonstrated that diamide, a biochemical that oxidizes glutathione and thus promotes protein glutathionylation, promoted reversible reductions in Mn-SOD activity when added to cultured rat renal tubular cells or when used to perfuse rat kidney *in vivo*.

PHYSIOLOGICAL SIGNIFICANCE OF MANGANESE SUPEROXIDE DISMUTASE ON KIDNEY DISEASE IN ANIMAL STUDIES

Lessons From Manganese Superoxide Dismutase Gene-Altered Mice

Studies featuring knockout (KO) of Mn-SOD activity via inactivating mutations or due to the complete elimination of Mn-SOD expression have been performed in mice. These manipulations have resulted in massive oxidative stress and neonatal death associated with cardiomyopathy, neurodegeneration, lipid accumulation in the liver and skeletal muscle, and metabolic acidosis (Li et al., 1995; Holley et al., 2012). There were no obvious alterations in mitochondrial structure in Mn-SOD gene-deleted ($^{-/-}$) mice; however, this gene deletion had a substantial impact on mitochondrial enzyme activity, including significant reductions in the activity of both succinate dehydrogenase and aconitase when compared with wild-type mice. By contrast, Lebovitz et al. (1996) previously generated a line of Mn-SOD knockout mice ($SOD2^{mlBCM}/SOD2^{mlBCM}$), in which the phenotype of mice is different from Mn-SOD knockout mice produced by other groups. Their mice survived longer than other group's mice and exhibited extensively swollen and damaged mitochondria within degenerating neurons and cardiac myocytes. These discrepancies on the phenotype of Mn-SOD knockout mice possibly depend on differences in the molecular constructs used to generate the deletions and in the genetic backgrounds on which the mutations were being bred. Williams et al. (1998) reported that Mn-SOD heterozygotes ($^{\pm}$) exhibited a 50% decrease in Mn-SOD activity, which was associated with impaired function and increased oxidative stress in the mitochondria in liver cells. Van Remmen et al. (2003) also reported increased and lifelong oxidative damage to DNA in liver, brain, and heart tissues of the Mn-$SOD^{\pm}$ mice; oxidative damage to DNA was associated with an increased incidence of cancer in these mice.

The physiological and pathological roles of Mn-SOD concerning renal function have been investigated using the heterozygous Mn-$SOD^{\pm}$ mice and with renal-specific Mn-SOD KO mice. Rodriguez-Iturbe et al. (2007) demonstrated oxidative stress and renal interstitial inflammation in a cohort of Mn-$SOD^{\pm}$ mice. These alterations were found to be compatible with accelerated renal senescence and the development of salt-sensitive hypertension. Additionally, Parajuli et al. (2011) created kidney-specific Mn-SOD KO mice from two different transgenic mouse lines. Specifically, "floxed" Mn-SOD mice (exon 3 of Mn-SOD allele flanked with LoxP sites in introns 2 and 3) were crossed with transgenic Ksp1.3/Cre (kidney Cre) mice.

The Ksp1.3/Cre transgenic mice expressed Cre recombinase specifically in distal tubules, collecting ducts, loops of Henle, ureteric buds, and the developing genitourinary tract of the kidney. As such, the impact of the Mn-SOD KO is limited to these specific parts of the kidney. Kidney-specific Mn-SOD KO mice exhibited normal renal function; however, the localized Mn-SOD KO resulted in modest renal damage, including tubular dilation, epithelial cell enlargement, the formation of casts within the tubular lumen, and the accumulation of nitrotyrosine-modified protein. These data indicate that Mn-SOD plays a crucial role in maintaining redox homeostasis in kidney mitochondria.

Kidney Disease Animal Model Studies

Mitochondrial oxidative stress associated with Mn-SOD dysfunction in the kidney is involved in the pathogenesis of several kidney diseases, including AKI and CKD, as well as in the AKI to CKD transition. Mn-SOD dysfunction has been associated with glomerular and tubulointerstitial fibrosis, inflammation, excess apoptosis of renal cells, and tubular cell damage, as one of the sources of ROS in the mitochondria (**Figure 3**). We focused on the change of Mn-SOD in the kidney, such as posttranslational modification, activity, and expression levels, in animal models of kidney disease, and showed the summary in **Table 1**.

Ischemia/Reperfusion-Induced Acute Kidney Injury

Increased oxidative stress is a well-known factor that contributes to renal ischemia/reperfusion (I/R) injury (Noiri et al., 2001). Mitochondria are remarkably sensitive to I/R injury, as I/R rapidly promotes the generation of $O_2^{\bullet-}$ and other ROS, including $ONOO^-$, within the injured mitochondria (Walker et al., 2000).

In the kidney of a rodent model of I/R AKI, Cruthirds et al. (2003) reported an acute increase in oxidative stress associated with increased levels of mitochondrial protein nitration, including Mn-SOD and cytochrome c, which resulted in Mn-SOD deactivation and mitochondrial dysfunction. Additionally, renal SIRT3 activity was reduced in response to I/R-induced AKI compared with activity observed among those in the control/sham-operated group. This was also accompanied by increased acetylation of both Mn-SOD and p53 (Ouyang et al., 2019). Taken together, these results suggest that inactivation of Mn-SOD induced by posttranslational modifications, such as nitration and acetylation, results in accelerated renal mitochondrial oxidative stress and contributes to the progression of I/R-induced AKI.

Le Clef et al. (2016) established a unilateral renal I/R model to study the associated mechanisms and therapies directed toward inhibiting the AKI to CKD transition in mice. Zhang et al. (2018) demonstrated that the mitochondrial complex-1 inhibitor, rotenone, slows the progression of AKI to CKD. By the mitochondrial complex-1 inhibitor, the expression of Mn-SOD and ATP synthase subunit β, mitochondrial DNA copy number, were restored, and inflammation and fibrosis were suppressed, in this model. These data suggest that mitochondrial $O_2^{\bullet-}$ may play a crucial role in promoting the pathogenesis associated with the transition from AKI to CKD.

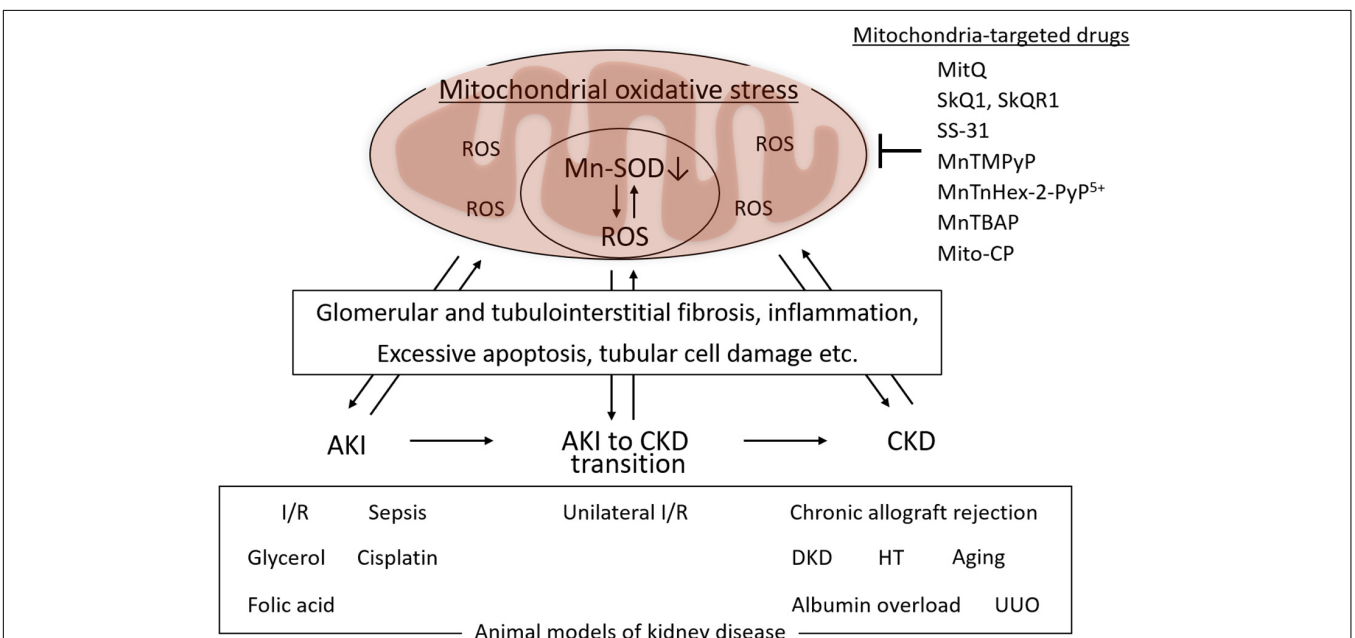


FIGURE 3 | Mitochondrial oxidative, manganese superoxide dismutase (Mn-SOD) dysfunction, and kidney disease. Mitochondrial oxidative stress associated with Mn-SOD dysfunction in the kidney is one of the key factors underlying the pathogenesis of kidney disease, including acute kidney injury (AKI), chronic kidney disease (CKD), as well as the AKI to CKD transition through glomerular and tubulointerstitial fibrosis, inflammation, excessive apoptosis of renal cells, and tubular cell damage. Several mitochondria-targeted drugs may improve the kidney disease through the improvement of mitochondrial oxidative stress. I/R, Ischemia/reperfusion; DKD, diabetic kidney disease; HT, hypertension; UUO, unilateral ureteral obstruction.

TABLE 1 | Change of renal manganese superoxide dismutase (Mn-SOD) in animal models of kidney disease.

Type of kidney disease	Animal models of kidney disease	Change of Mn-SOD in the kidney	References
AKI	**I/R-induced AKI models**		
	I/R mice	Tyrosine nitration of Mn-SOD ↑	Cruthirds et al., 2003
		Acetylated Mn-SOD ↑	Ouyang et al., 2019
	Sepsis-associated AKI models		
	CLP mice	Mn-SOD activity ↓	Patil et al., 2014
		Mn-SOD expression/activity ↓	Chen G. D. et al., 2018
	CLP rats	Acetylated Mn-SOD ↑	Xu, 2016
	Other AKI models		
	Glycerol-induced AKI rats	Mn-SOD expression ↓	Homsi et al., 2011
	Cisplatin-induced AKI mice	Mn-SOD expression ↓	Rattanavich et al., 2013
	Folic acid-induced AKI mice	Mn-SOD expression ↓	Zhang et al., 2019
AKI to CKD transition	Unilateral I/R mice	Mn-SOD expression ↓	Zhang et al., 2018
CKD	**Chronic allograft rejection and associated nephropathy models**		
	Chronic allograft rejection of rats	Tyrosine nitration of Mn-SOD ↑	MacMillan-Crow et al., 1996, 2001
	DKD models		
	db/db mice	Tyrosine nitration of Mn-SOD ↑	Kitada et al., 2011
		Mn-SOD expression ↓	Lu et al., 2013; Hou et al., 2018
	STZ-induced diabetic Apo $E^{-/-}$ mice	Tyrosine nitration of Mn-SOD ↑	Xu et al., 2006
		Mn-SOD expression ↓	Yi et al., 2011
	Zucker diabetic fatty rats	Acetylated Mn-SOD ↑	Ogura et al., 2018
	STZ-induced diabetic mice	Mn-SOD expression ↓	Spencer et al., 2018; Li et al., 2019
	A high-sugar/high-fat or cholesterol diet with STZ-induced diabetic rats	Mn-SOD expression ↓	Onozato et al., 2004; Chen P. et al., 2018; Zayed et al., 2018
	BTBR ob/ob mice	Mn-SOD expression ↓	Morigi et al., 2020
	Diabetic SHRs	Mn-SOD expression ↓	Tang et al., 1997
	KK/Ta-Akita mice	Mn-SOD expression ↑	Fujita et al., 2009
	Hypertension-related kidney injury models		
	Angiotensin II-induced hypertension rats	Tyrosine nitration of Mn-SOD ↑	Guo et al., 2003
	SHRs treated with the NOS inhibitor	Mn-SOD expression ↑, Mn-SOD activity ↑	Majzunova et al., 2019
	SHRs fed high-fat diet	Mn-SOD expression ↓	Chung et al., 2012
	Aging-related kidney injury models		
	24 months old mice (vs. 12 months old mice)	Mn-SOD expression ↓	Lim et al., 2012
	$Klotho^{-/-}$ mice	Mn-SOD expression ↓	Kimura et al., 2018
	16 months old mice (vs. 8 weeks old mice)	Mn-SOD expression ↓	Hou et al., 2016
	20 months old mice (vs. 3 months old mice)	Mn-SOD expression ↓	Teissier et al., 2019
	52 weeks old rats (vs. 13 weeks old rats)	Mn-SOD expression ↑	Gomes et al., 2009
	Other CKD models		
	Albumin-overload mice	Mn-SOD expression ↓	Zhuang et al., 2015; Jia et al., 2016
	UUO mice	Mn-SOD expression ↓	Li et al., 2017; Liu et al., 2017

AKI, Acute kidney disease; CKD, chronic kidney disease; I/R, ischemic/reperfusion; CLP, cecal ligation and puncture; STZ, streptozotocin; SHRs, spontaneously hypertensive rats; UUO, unilateral ureteral obstruction.

Sepsis-Associated Acute Kidney Injury

Oxidative stress plays a crucial role in the development of mitochondrial dysfunction in response to septic AKI (Schrier and Wang, 2004). Patil et al. (2014) reported a decrease in renal Mn-SOD activity in association with the experiment in sepsis mice, which led to mitochondrial oxidative stress. Treatment with Mito-TEMPO, a mitochondrial-targeted mimetic of SOD, resulted in improvements in sepsis-induced AKI, which were directly associated with increased activation of Mn-SOD and decreased mitochondrial oxidative stress. Additionally, Chen G. D. et al., 2018 demonstrated that renal Mn-SOD expression and activity were reduced in sepsis-induced AKI and that insulin therapy reversed mitochondrial dysfunction via the suppression of the oxidative stress associated with upregulation of Mn-SOD. Furthermore, Xu (2016) found that SIRT1/SIRT3 activity was reduced in the renal tubular epithelial cells of rats with sepsis-associated AKI. This finding was accompanied by increasing levels of acetylated Mn-SOD, swollen mitochondria, and elevated levels of cellular apoptosis. Interestingly, activation of SIRT1 via treatment with resveratrol

partially restored SIRT3 activity, which led to improved mitochondrial function and reduced levels of apoptosis. Taken together, these data indicate that Mn-SOD dysfunction and associated mitochondrial oxidative stress are both closely linked to mechanisms promoting disease pathogenesis in sepsis-related AKI.

Other Acute Kidney Injury Models

Glycerol-induced AKI results in increased expression of phosphorylated p53 in renal tissues in association with elevated levels of tubular cell apoptosis and oxidative stress due to decreased expression of antioxidative enzymes, including Mn-SOD (Homsi et al., 2011). Treatment with the p53 inhibitor, pifithrin-α, reversed features of glycerol-induced renal injury, including tubular cell apoptosis and oxidative stress via its capacity to restore Mn-SOD expression in the kidney (Homsi et al., 2011).

Oxidative stress and mitochondrial dysfunction related to ATP depletion have been observed in response to cisplatin-induced cellular injury in the kidney (Arany and Safirstein, 2003). Rattanavich et al. (2013) reported that cisplatin treatment results in diminished expression of renal Mn-SOD via the enhanced expression of phospho-p66ShcA and phospho-Foxo3A in studies featuring heterozygous p66ShcA ($^{\pm}$) mice. Specifically, increased expression of phospho-p66ShcA and phospho-Foxo3A was detected in the renal tissue of wild-type mice treated with cisplatin in association with reduced expression of both Mn-SOD and catalase. By contrast, renal tissue from cisplatin-treated p66ShcA$^{\pm}$ mice revealed attenuated phosphorylation of p66ShcA and Foxo3a, together with a restored expression of Mn-SOD and catalase.

In the folic acid (FA)-induced AKI model, mitochondrial DNA copy number and expression of mitochondrial cytochrome c oxidase subunit 1, mitochondrial NADH dehydrogenase subunit 1, and Mn-SOD in the kidney were all reduced. These findings implicate mitochondrial dysfunction and oxidative stress in the pathogenesis of FA-induced AKI (Zhang et al., 2019). However, pretreatment with rotenone aggravated the renal injury, mitochondrial damage, and oxidative stress in this mouse model of AKI.

Chronic Renal Allograft Rejection

Chronic allograft rejection is currently the most important cause of renal transplant failure (Shrestha and Haylor, 2016). At the tissue level, increased oxidative stress observed in response to I/R may contribute to chronic allograft rejection and associated nephropathy. MacMillan-Crow et al. (1996, 2001) previously reported that endogenous tyrosine nitration and inactivation of Mn-SOD were observed in both human recipients and rat models of chronic renal allograft rejection. These data suggest that deactivation of Mn-SOD by $ONOO^{-}$ results in a progressive increase in the production of $ONOO^{-}$, leading to irreversible oxidative injury to mitochondria in the renal allograft.

Diabetic Kidney Disease

Diabetic kidney disease (DKD) is among the main causes of ESRD and an independent risk factor for CVD (Ninomiya et al., 2009). Mitochondrial dysfunction has been linked to the pathogenesis of DKD, and mitochondrial oxidative stress is closely associated with mitochondrial dysfunction (Sharma, 2017; Xu et al., 2020). Dysfunction or decreased expression of Mn-SOD is one of the major factors associated with mitochondrial oxidative stress in diabetic kidney and, as such, with the pathogenesis of DKD.

The increased detection of nitrotyrosine in proximal tubules in renal biopsy samples from diabetic patients compared with non-diabetic control suggested that oxidant injury within the proximal tubules may play a critical role in the pathogenesis of DKD (Thuraisingham et al., 2000). Reduction of Mn-SOD activity in the kidney due to posttranslational modifications and oxidative stress has also been shown in several diabetic animal models. We previously demonstrated that mitochondrial oxidative stress is enhanced in the kidney of diabetic db/db mice when compared with kidneys from non-diabetic db/m controls. These observations were linked to tyrosine nitration of Mn-SOD and the associated reduction in enzyme activity (Kitada et al., 2011). Administration of resveratrol reversed the level of mitochondrial oxidative stress by limiting the extent of nitration, thereby increasing Mn-SOD activity in the diabetic kidney (Kitada et al., 2011). Similarly, in the kidneys of STZ-induced diabetic apolipoprotein $E^{-/-}$ mice, a decrease in Mn-SOD activity was associated with an increase in Mn-SOD Tyr-34 nitration (Xu et al., 2006). Antagonists of thromboxane A_2 receptors limited diabetes-induced renal injury, which was also accompanied by a reduction of tyrosine nitration (Xu et al., 2006). Additionally, we found that mitochondrial oxidative stress was induced by a reduction in Mn-SOD activity via increased acetylation at K68; this finding was associated with a decreased intracellular NAD^{+}/NADH ratio and decreased SIRT3 activity accompanied by increased expression of CD38, a NAD-degrading enzyme, in the kidneys of Zucker diabetic fatty rats (Ogura et al., 2018). Additionally, administration of the CD38 inhibitor, apigenin, resulted in improved mitochondrial oxidative stress through SIRT3 activation and Mn-SOD deacetylation (Ogura et al., 2020).

The expression of Mn-SOD is reduced in several diabetic animal models, including STZ-induced type 1 diabetes (Spencer et al., 2018; Li et al., 2019), a high-sugar/high-fat or cholesterol diet with STZ-induced diabetes (Onozato et al., 2004; Chen P. et al., 2018; Zayed et al., 2018), Apo $E^{-/-}$ mice with STZ-induced diabetes (Yi et al., 2011), db/db mice (Lu et al., 2013; Hou et al., 2018), BTBR ob/ob mice (Morigi et al., 2020), and diabetic spontaneously hypertensive rats (SHR) (Tang et al., 1997). This has been associated with increased oxidative stress in kidney mitochondria. However, the diabetic KK/Ta-Akita mice feature the downregulation of cytosolic Cu/Zn-SOD and extracellular Cu/Zn-SOD, but not mitochondrial Mn-SOD, in the kidney (Fujita et al., 2009). Additionally, Dugan et al. (2013) demonstrated no increased renal disease in STZ-induced diabetes in heterozygous Mn-SOD$^{\pm}$ mice over that observed in STZ-induced WT mice.

Hypertension-Related Kidney Injury

Oxidative stress in blood vessels and the kidney due to chronic hypertension may result from the dysregulated responses of

several vasoconstrictor mechanisms, including those associated with angiotensin II stimulation or with dysfunction of nitric oxide synthase (eNOS). Angiotensin II-induced hypertension promotes an increase in $O_2^{\bullet-}$ generation via the activation of NADPH oxidase (Wang et al., 2001; Onozato et al., 2002). The antioxidant response is impaired in hypertensive animals, which results in oxidative stress followed by the development of end-organ damage, including ESRD. Guo et al. (2003) demonstrated that angiotensin II-induced hypertension in rats was accompanied by increased tyrosine nitration and thus deactivation of Mn-SOD that serves to augment oxidative stress in the kidney. Activation of NADPH oxidase also induces the production of $O_2^{\bullet-}$, and $ONOO^-$ also may induce eNOS uncoupling, leading to overproduction of $O_2^{\bullet-}$ as opposed to NO.

Protein nitration was increased in the renal cortex of SHRs treated with the NOS inhibitor, L-NAME (Majzunova et al., 2019). Renal expression of Mn-SOD was increased in SHR in response to treatment with L-NAME, although total SOD activity remained unchanged (Majzunova et al., 2019). These data suggest that the impaired antioxidant response combined with a deficiency in NO may promote oxidative damage of proteins in the kidney via the production of $ONOO^-$ in SHRs. By contrast, SHRs that fed a high-fat diet (HFD) developed renal lipotoxicity, insulin resistance, and hypertension in association with a decrease in PPARα expression. This leads to Akt activation, increased FoxO3a phosphorylation, suppression of the PGC-1α-ERR-1α pathway, reduced expression of Mn-SOD, and increased oxidative stresses (Chung et al., 2012).

Aging-Related Kidney Dysfunction

Aging-related changes lead to the functional decline of several organs, including the kidney. Oxidative stress, particularly mitochondrial oxidative stress, has been recognized as one of the contributors to the aging process (Vlassara et al., 2009; Kume et al., 2010). Reduction or dysfunction of Mn-SOD-mediated mitochondrial oxidative stress may be involved in the pathogenesis associated with the aging kidney.

Lim et al. (2012) reported increased oxidative stress in the kidneys of aged (24 months old) mice compared with that observed among younger mice. This finding was associated with a reduction in Cu/Zn-SOD and Mn-SOD expression in the kidney, which may be mediated by decreases in SIRT1, PGC-1α, estrogen-related receptor (ERR)-1α, PPARα, and Klotho. Klotho has been identified as an antiaging gene that is primarily expressed in the kidney, parathyroid, and choroid plexus (Kuro-o et al., 1997); decreased Klotho expression has been correlated with kidney disease. Klotho exerts resistance against oxidative stress, possibly via the activation of FoxO transcription factors, which lead to increased expression of antioxidative enzymes, including Mn-SOD (Yamamoto et al., 2005). $Klotho^{-/-}$ mice exhibit enhanced nitrotyrosine levels and reduced levels of antioxidative enzymes, including Mn-SOD in the kidney (Kimura et al., 2018). Additionally, Hou et al. (2016) demonstrated that the production of endogenous hydrogen sulfide (H_2S) in the aging kidney is insufficient. Exogenous H_2S can reduce renal oxidative stress in aging mice via a mechanism that involves enhanced nuclear translocation of Nrf2 and increased expression of antioxidative enzymes, including Mn-SOD (Hou et al., 2016). Moreover, the endogenous advanced glycated end products (AGEs)/receptor for AGEs (RAGE) axis mediates factors associated with the aging process in many tissues and organs, including the kidney. Teissier et al. (2019) showed that older (20 months old) WT mice exhibited renal inflammation and oxidative stress with reduced expression of Mn-SOD compared with 3-month-old counterparts. By contrast, 20-month-old $RAGE^{-/-}$ mice were characterized by lower inflammation and elevated expression levels of Mn-SOD and SIRT1 in the kidney compared with their 20-month-old WT counterparts (Teissier et al., 2019).

By contrast, Gomes et al. (2009) detected increased levels of NADPH oxidase subunits and H_2O_2 in aged Wistar–Kyoto rats (52 weeks old) compared with those in young rats, although aged rats show no renal functional impairment or marked elevation in blood pressure. The aged rats did exhibit increased expression of antioxidative enzymes, including Mn-SOD, extracellular Cu/Zn-SOD, and catalase (Gomes et al., 2009), and these findings reveal that increases in antioxidant defenses may be useful for counteracting the damaging impact of oxidative stress.

Other Chronic Kidney Disease Animal Models

Albumin overload is a well-known model of renal tubulointerstitial injury caused by inflammation and fibrosis (Eddy, 1989). In the kidney of albumin-overload mice, Mn-SOD expression is reduced, thus promoting mitochondrial oxidative stress and inflammation. This condition can be improved by treatment with an Mn-SOD mimic [Mn (III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP)] (Zhuang et al., 2015; Jia et al., 2016).

Unilateral ureteral obstruction (UUO) in a rodent model generates progressive renal fibrosis (Chevalier et al., 2009). Oxidative stress and inflammation are closely associated with the pathogenesis of UUO-induced renal fibrosis (Chevalier et al., 2009). In the kidney of UUO mice, the expression of antioxidative enzymes, including Mn-SOD, is reduced, resulting in mitochondrial oxidative stress, inflammation, and tubular epithelial–mesenchymal transition (EMT; Li et al., 2017; Liu et al., 2017). This condition can be improved by treatment with MnTBAP (Li et al., 2017; Liu et al., 2017).

PHYSIOLOGICAL SIGNIFICANCE OF MANGANESE SUPEROXIDE DISMUTASE ON KIDNEY DISEASE IN HUMAN STUDIES

Manganese Superoxide Dismutase Gene Polymorphisms on Diabetic Kidney Disease

Ala16Val (rs4880C/T) is a functional Mn-SOD SNP polymorphism in exon 2 of *sod2* gene. The substitution of

C to T (GCT to GTT), resulting in the conversion from alanine (A) to valine (V), induces a conformational change in the mitochondrial targeting sequence. This results in less efficient transport of Mn-SOD into the mitochondrial matrix and decreases antioxidant activity within the mitochondria (Shimoda-Matsubayashi et al., 1996; Sutton et al., 2003). The relationship of this Mn-SOD gene polymorphism with DKD has been studied widely.

Previous reports revealed an association between the Mn-SOD gene polymorphism and the risk for developing DKD. Möllsten et al. (2007) reported that VV genotype was associated with an increased risk of diabetic nephropathy among Finnish and Swedish patients with type 1 diabetes. Nomiyama et al. (2003) identified the VV genotype with significantly higher frequency in the diabetic patients with nephropathy compared with the AA or VA genotype; however, this was unrelated to the etiology of type 2 diabetes in Japanese patients. By contrast, Houldsworth et al. (2015) demonstrated that the CC (alanine/alanine) genotype in type 1 diabetic patients might protect against diabetic nephropathy. According to another study on Korean type 2 diabetic patients, the alanine-encoding allele was detected at significantly lower frequency among patients with nephropathy who have micro- or macroalbuminuria compared with those patients without nephropathy (Lee et al., 2006). Liu et al. (2009) showed that the AA and VA genotypes were independent factors that protected against the development of nephropathy in Chinese type 2 diabetic patients. Ascencio-Montiel et al. (2013) demonstrated that in type 2 diabetes patients from Mexico, the frequency of the TT (valine/valine) genotype was 6.7% higher in patients with macroalbuminuria than in those in the normoalbuminuria group; the CC (alanine/alanine) genotype was associated with a lower risk of macroalbuminuria than the TT (valine/valine) genotype (Tian et al., 2011). Moreover, in a meta-analysis that included five studies focusing on type 2 diabetic patients, the alanine-encoding allele was associated with a reduced risk of macroalbuminuria (CR 0.65, 95% CI 0.52–0.80) compared with the valine-encoding allele (Tian et al., 2011). Likewise, a meta-analysis that included 17 published articles with both type 1 and 2 diabetic patients revealed a significant association of the alanine-encoding allele with a reduced risk of diabetic microvascular complications and nephropathy in the dominant model (OR 0.788, 95% CI 0.680–0.914 and OR 0.801, 95% CI 0.664–0.967, respectively) (Ascencio-Montiel et al., 2013).

Membranous Nephropathy

Membranous nephropathy (MN) is a leading cause of nephrotic syndrome in adults. Glomerular damage is induced by subepithelial immune deposits that consist mainly of IgG_4 and complement components C5b-9. Several podocyte proteins, including aldose reductase (AR) and Mn-SOD, have been identified as renal antigens associated with the pathogenesis of MN (Prunotto et al., 2010). Confocal and immunoelectron microscopy (IEM) displayed co-localization of anti-AR and anti-Mn-SOD IgG_4 and C5b-9 in electron-immune deposits in podocytes. Additionally, Murtas et al. (2012) demonstrated that the levels of serum IgG_4 directed against AR and Mn-SOD in patients with MN are higher in MN than those in any of the other proteinuria-associated glomerulonephritides, including focal glomerulonephropathy and IgA nephritis.

MITOCHONDRIA-TARGETED THERAPIES FOR SCAVENGING $O_2^{\bullet-}$

Mitochondria are both sources and targets of ROS. The dysfunction of mitochondria due to oxidative stress may be the causal factor for kidney disease. As such, it will be necessary to develop pharmacological methods aimed at reducing oxidative damage to mitochondria and their components, including Mn-SOD. Several molecules that accumulate within the mitochondria and can scavenge ROS have been developed to address this issue. The beneficial effect of mitochondria-targeted drugs for kidney injury has been shown in several animal models (**Table 2**). However, those drugs are not delivered to only the kidney; therefore, the risk of adverse effects in systemic administration remains. To address this issue, it is necessary to develop the drugs that specifically deliver to the kidney.

MitoQ

MitoQ is a derivative of ubiquinone conjugated to triphenylphosphonium (TTP), a lipophilic cation that enables this molecule to enter and accumulate within the mitochondria via an electrochemical gradient (Smith et al., 2003; Smith and Murphy, 2010). In the mitochondrial matrix, MitoQ is reduced to the active antioxidant form, ubiquinol, by the respiratory chain, which serves to protect the mitochondria and their components from oxidative damage (Smith et al., 2003; Smith and Murphy, 2010). Additionally, MitoQ is cleared rapidly from the plasma after intravenous administration and accumulates within the kidney (Porteous et al., 2010; Smith and Murphy, 2010). Previous animal studies have revealed the beneficial effects of MitoQ in association with various kidney disorders, including I/R-induced AKI (Dare et al., 2015; Kezic et al., 2016), sepsis-induced AKI (Lowes et al., 2008), cisplatin-induced kidney injury (Mukhopadhyay et al., 2012), DKD (Chacko et al., 2010; Xiao et al., 2017; Han et al., 2018), and polycystic kidney disease (Ishimoto et al., 2017).

SkQ1 and SkQR1

SkQ1 is a compound of plastoquinonyl-decyl-triphenylphosphonium in which ubiquinone was replaced by plastoquinone (Antonenko et al., 2008). SkQR1 is also a mitochondria-targeted compound and a conjugate of a positively charged rhodamine molecule with plastoquinone (Antonenko et al., 2008). SkQs, including SkQ1 and SkQR1, penetrate planar phospholipid membranes, and as such, they can accumulate in mitochondria (Antonenko et al., 2008). Administration of SkQ1 to mitochondrial DNA mutator mice resulted in diminished renal oxidative stress and mitochondrial dysfunction (Shabalina et al., 2017). In experimental rats with I/R injury, SkQR1 treatment

TABLE 2 | Mitochondria-targeted drugs and animal models of kidney disease.

Mitochondria-targeted drugs	Antioxidative moiety	Animal models of kidney disease showing the benefit of mitochondria-targeted drugs	References
MitoQ	Ubiquinone	I/R-induced AKI mice	Dare et al., 2015
		Sepsis-associated AKI rats	Lowes et al., 2008
		Cisplatin-induced AKI mice	Mukhopadhyay et al., 2012
		Ins2(+/)$^{-}$(AkitaJ) mice	Chacko et al., 2010
		db/db mice	Xiao et al., 2017; Han et al., 2018
		ADPKD model mice (Ksp-Cre PKD1$^{flox/flox}$) and rats (Han:SPRD Cy/+)	Ishimoto et al., 2017
SkQ1	Plastoquinone	mtDNA mutator mice	Shabalina et al., 2017
SkQR1		I/R-induced AKI rats	Plotnikov et al., 2011
		Gentamicin-induced AKI rats	Jankauskas et al., 2012
SS-31	Tyr or Dmt (2′,6′-dimethyltyrosine) residues	I/R injury rats	Szeto et al., 2011
		PTRA-induced I/R pig	Eirin et al., 2012
MnTMPyP	Manganese metalloporphyrin	I/R injury of rats	Liang et al., 2009
MnTnHex-2-PyP^{5+}		I/R-induced AKI rats	Dorai et al., 2011
MnTBAP		Albumin-overload model mice	Zhuang et al., 2015; Jia et al., 2016
		UUO model mice	Liu et al., 2017
Mito-CP	Nitroxide CP	Cisplatin-induced AKI mice	Mukhopadhyay et al., 2012

MnTMPyP, MnP manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin; MnTnHex-2-PyP^{5+}, Mn (III) meso-tetrakis (N-n-hexylpyridinium-2-yl) porphyrin; MnTBAP, Mn (III) tetrakis (4-benzoic acid) porphyrin chloride; CP, carboxy-proxyl; I/R, ischemic/reperfusion; AKI, acute kidney disease; ADPKD, autosomal dominant polycystic kidney disease; PTRA, percutaneous transluminal renal angioplasty; UUO, unilateral ureteral obstruction.

normalized the ROS levels in kidney mitochondria, decreased blood urea nitrogen (BUN) and creatinine levels, and reduced disease-associated mortality, compared with what was observed among the rats without treatment (Plotnikov et al., 2011). Additionally, SkQR1 was also effective for the treatment of gentamicin-induced AKI (Jankauskas et al., 2012).

SS-31

The Szeto–Schiller (SS)-31 peptide, also known as Bendavia, may optimize the mitochondrial phospholipid cardiolipin microdomains. This may lead to reduced electron leak from the inner membrane (Brown et al., 2013). In an experimental model of I/R injury in the kidney, treatment with SS-31 attenuated renal oxidative stress prevented tubular cell apoptosis and necrosis, and reduced inflammation (Szeto et al., 2011). Rapid ATP recovery in mitochondria secondary to administration of SS-31 led to the protection of microvascular endothelial cells, reduced microvascular congestion, and, thus, better reflow to the medulla. In an experimental pig model featuring percutaneous transluminal renal angioplasty, administration of SS-31 resulted in decreased microvascular rarefaction, apoptosis, oxidative stress, tubular injury, and fibrosis, which were all associated with preserved mitochondrial biogenesis in the kidney after reperfusion (Eirin et al., 2012).

Manganese Superoxide Dismutase Mimics

Mn-SOD mimics are stoichiometric scavengers of $O_2^{\bullet-}$ and accumulate in mitochondria depending on their positive charge and lipophilicity (Miriyala et al., 2012). Among the cationic metalloporphyrins, the Mn porphyrins (MnP) are the most potent Mn-SOD mimics; these molecules have been designed and optimized for accumulation in the mitochondria where they mimic the action of the Mn-SOD catalytic site (Miriyala et al., 2012). The MnP manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP) functions as a peroxynitrite scavenger. Administration of MnTMPyP resulted in diminished lipid peroxidation and reduced nitrotyrosine content in the proximal tubular area in association with reductions in caspase-3 activation and tubular epithelial cell damage after I/R injury in rats (Liang et al., 2009). Similarly, administration of Mn (III) meso-tetrakis (N-n-hexylpyridinium-2-yl) porphyrin (MnTnHex-2-PyP^{5+}) or Mn (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBAP) also resulted in protection against the deleterious effects associated with I/R-induced AKI (Dorai et al., 2011) as well as in the albumin-overload kidney injury (Zhuang et al., 2015; Jia et al., 2016) and the UUO model (Liu et al., 2017). Moreover, mitochondria-targeted carboxy-proxyl (Mito-CP) is a five-membered nitroxide CP conjugated to the TTP cation. Administration of Mito-CP resulted in improvements in parameters associated with cisplatin-induced AKI, including kidney dysfunction, renal inflammation, and tubular cell apoptosis, which were equivalent to those of MitoQ (Mukhopadhyay et al., 2012).

CONCLUSION AND PERSPECTIVES

Mitochondrial oxidative stress is induced by an imbalance between ROS production and scavenging; the scavenging

function may be impaired or destroyed due to dysfunction of antioxidative defense in mitochondria. Mitochondrial oxidative stress contributes to the pathogenesis of kidney disease, including AKI, CKD, and the AKI to CKD transition, and mitochondria-targeted drugs may suppress the onset or progression of kidney disease (**Figure 3**). Mn-SOD-associated dysfunction is one of the main factors underlying defective antioxidative defense in mitochondria. Mn-SOD dysfunction results in a net increase in ROS and generates a vicious cycle in which mitochondrial oxidative stress is amplified, as Mn-SOD then becomes a target of dysfunctional oxidative modification. An agent with the capacity to scavenge excess ROS in mitochondria would lead to a break in this vicious cycle, serving to preserve Mn-SOD and mitochondrial function and consequently suppress kidney disease. Although Mn-SOD may become dysfunctional in kidney disease, it remains unclear whether mitochondrial oxidative stress due to only Mn-SOD dysfunction is the primary contributor to the pathogenesis of kidney disease. Redox state in mitochondria is regulated by not only Mn-SOD but also other antioxidative enzymes, including Cu/Zn-SOD, GPx, and the PRx/TRx system. However, as Mn-SOD catalyzes the first step in ROS scavenging in mitochondria, treatments targeted at supporting Mn-SOD integrity and function may lead to effective treatments to prevent the onset and progression of kidney disease.

AUTHOR CONTRIBUTIONS

MK designed, wrote, and edited the manuscript. JX, YO, IM, and DK contributed to the discussion. MK was the guarantor of this work. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported in part by the Japan–China Sasakawa Medical Fellowship to JX.

REFERENCES

Antonenko, Y. N., Avetisyan, A. V., Bakeeva, L. E., Chernyak, B. V., Chertkov, V. A., Domnina, L. V., et al. (2008). Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. Cationic plastoquinone derivatives: synthesis and in vitro studies. *Biochemistry* 73, 1273–1287. doi: 10.1134/s0006297908120018

Arany, I., and Safirstein, R. L. (2003). Cisplatin nephrotoxicity. *Semin. Nephrol.* 23, 460–464.

Ascencio-Montiel, I. J., Parra, E. J., Valladares-Salgado, A., Gómez-Zamudio, J. H., Kumate-Rodriguez, J., Escobedo-de-la-Peña, J., et al. (2013). SOD2 gene Val16Ala polymorphism is associated with macroalbuminuria in Mexican type 2 diabetes patients: a comparative study and meta-analysis. *BMC Med. Genet.* 14:110. doi: 10.1186/1471-2350-14-110

Barra, D., Schinina, M. E., Simmaco, M., Bannister, J. V., Bannister, W. H., Rotilio, G., et al. (1984). The primary structure of human liver manganese superoxide dismutase. *J. Biol. Chem.* 259, 12595–12601.

Beckman, J. S., and Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol.* 271, C1424–C1437.

Borgstahl, G. E., Parge, H. E., Hickey, M. J., Beyer, W. F. Jr., Hallewell, R. A., and Tainer, J. A. (1992). The structure of human mitochondrial manganese superoxide dismutase reveals a novel tetrameric interface of two 4-helix bundles. *Cell* 71, 107–118. doi: 10.1016/0092-8674(92)90270-m

Borrello, S., and Demple, B. (1997). NF Kappa B-independent transcriptional induction of the human manganous superoxide dismutase gene. *Arch. Biochem. Biophys.* 348, 289–294. doi: 10.1006/abbi.1997.0355

Brown, D. A., Sabbah, H. N., and Shaikh, S. R. (2013). Mitochondrial inner membrane lipids and proteins as targets for decreasing cardiac ischemia/reperfusion injury. *Pharmacol. Ther.* 140, 258–266. doi: 10.1016/j.pharmthera.2013.07.005

Candas, D., Fan, M., Nantajit, D., Vaughan, A. T., Murley, J. S., Woloschak, G. E., et al. (2013). CyclinB1/Cdk1 phosphorylates mitochondrial antioxidant MnSOD in cell adaptive response to radiation stress. *J. Mol. Cell Biol.* 5, 166–175. doi: 10.1093/jmcb/mjs062

Castellano, I., Cecere, F., De Vendittis, A., Cotugno, R., Chambery, A., Di Maro, A., et al. (2009). Rat mitochondrial manganese superoxide dismutase: amino acid positions involved in covalent modifications, activity, and heat stability. *Biopolymers* 91, 1215–1226. doi: 10.1002/bip.21208

Chacko, B. K., Reily, C., Srivastava, A., Johnson, M. S., Ye, Y., Ulasova, E., et al. (2010). Prevention of diabetic nephropathy in Ins2(+/)- (AkitaJ) mice by the mitochondria-targeted therapy MitoQ. *Biochem. J.* 432, 9–19. doi: 10.1042/bj20100308

Che, R., Yuan, Y., Huang, S., and Zhang, A. (2014). Mitochondrial dysfunction in the pathophysiology of renal diseases. *Am. J. Physiol. Renal Physiol.* 306, F367–F378.

Chen, G. D., Zhang, J. L., Chen, Y. T., Zhang, J. X., Wang, T., and Zeng, Q. Y. (2018). Insulin alleviates mitochondrial oxidative stress involving upregulation of superoxide dismutase 2 and uncoupling protein 2 in septic acute kidney injury. *Exp. Ther. Med.* 15, 3967–3975.

Chen, P., Shi, X., Xu, X., Lin, Y., Shao, Z., Wu, R., et al. (2018). Liraglutide ameliorates early renal injury by the activation of renal FoxO1 in a type 2 diabetic kidney disease rat model. *Diabetes Res. Clin. Pract.* 137, 173–182. doi: 10.1016/j.diabres.2017.09.006

Chen, Y., Zhang, J., Lin, Y., Lei, Q., Guan, K. L., Zhao, S., et al. (2011). Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. *EMBO Rep.* 12, 534–541. doi: 10.1038/embor.2011.65

Cherry, A. D., Suliman, H. B., Bartz, R. R., and Piantadosi, C. A. (2014). Peroxisome proliferator-activated receptor gamma co-activator 1-α as a critical co-activator of the murine hepatic oxidative stress response and mitochondrial biogenesis in Staphylococcus aureus sepsis. *J. Biol. Chem.* 289, 41–52. doi: 10.1074/jbc.m113.512483

Chevalier, R. L., Forbes, M. S., and Thornhill, B. A. (2009). Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney Int.* 75, 1145–1152. doi: 10.1038/ki.2009.86

Chung, H. W., Lim, J. H., Kim, M. Y., Shin, S. J., Chung, S., Choi, B. S., et al. (2012). High-fat diet-induced renal cell apoptosis and oxidative stress in spontaneously hypertensive rat are ameliorated by fenofibrate through the PPARα-FoxO3a-PGC-1α pathway. *Nephrol. Dial. Transplant.* 27, 2213–2225. doi: 10.1093/ndt/gfr613

Creagan, R., Tischfield, J., Ricciuti, F., and Ruddle, F. H. (1973). Chromosome assignments of genes in man using mouse-human somatic cell hybrids: mitochondrial superoxide dismutase (indophenol oxidase-B, tetrameric) to chromosome 6 Humangenetik. *Redox Biol.* 20, 203–209. doi: 10.1007/bf00385731

Cruthirds, D. L., Novak, L., Akhi, K. M., Sanders, P. W., Thompson, J. A., and MacMillan-Crow, L. A. (2003). Mitochondrial targets of oxidative stress during renal ischemia/reperfusion. *Arch. Biochem. Biophys.* 412, 27–33. doi: 10.1016/s0003-9861(03)00039-0

Dare, A. J., Bolton, E. A., Pettigrew, G. J., Bradley, J. A., Saeb-Parsy, K., and Murphy, M. P. (2015). Protection against renal ischemia-reperfusion injury in vivo by the mitochondria targeted antioxidant MitoQ. *Redox Biol.* 5, 163–168. doi: 10.1016/j.redox.2015.04.008

Dhar, S. K., Xu, Y., Chen, Y., and St. Clair, D. K. (2006). Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide

dismutase gene expression. *J. Biol. Chem.* 281, 21698–21709. doi: 10.1074/jbc.m601083200

Dhar, S. K., Xu, Y., and St. Clair, D. K. (2010). Nuclear factor kappaB- and specificity protein 1-dependent p53-mediated bi-directional regulation of the human manganese superoxide dismutase gene. *J. Biol. Chem.* 285, 9835–9846. doi: 10.1074/jbc.m109.060715

Ding, Y., Yang, H., Wang, Y., Chen, J., Ji, Z., and Sun, H. (2017). Sirtuin 3 is required for osteogenic differentiation through maintenance of PGC-1α-SOD2-mediated regulation of mitochondrial function. *Int. J. Biol. Sci.* 13, 254–264. doi: 10.7150/ijbs.17053

Dorai, T., Fishman, A. I., Ding, C., Batinic-Haberle, I., Goldfarb, D. S., and Grasso, M. (2011). Amelioration of renal ischemia-reperfusion injury with a novel protective cocktail. *J. Urol.* 186, 2448–2454. doi: 10.1016/j.juro.2011.08.010

Drane, P., Bravard, A., Bouvard, V., and May, E. (2001). Reciprocal down-regulation of p53 and SOD2 gene expression-implication in p53 mediated apoptosis. *Oncogene* 20, 430–439. doi: 10.1038/sj.onc.1204101

Dugan, L. L., You, Y. H., Ali, S. S., Diamond-Stanic, M., Miyamoto, S., DeCleves, A. E., et al. (2013). AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. *J. Clin. Invest.* 123, 4888–4899. doi: 10.1172/jci66218

Eckardt, K. U., Coresh, J., Devuyst, O., Johnson, R. J., and Köttgen, A. (2013). Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet* 382, 158–169. doi: 10.1016/s0140-6736(13)60439-0

Eddy, A. A. (1989). Interstitial nephritis induced by protein-overload proteinuria. *Am. J. Pathol.* 135, 719–733.

Eirin, A., Li, Z., Zhang, X., Krier, J. D., Woollard, J. R., Zhu, X. Y., et al. (2012). A mitochondrial permeability transition pore inhibitor improves renal outcomes after revascularization in experimental atherosclerotic renal artery stenosis. *Hypertension* 60, 1242–1249. doi: 10.1161/hypertensionaha.112.199919

Ennen, M., Minig, V., Grandemange, S., Touche, N., Merlin, J. L., Besancenot, V., et al. (2011). Regulation of the high basal expression of the manganese superoxide dismutase gene in aggressive breast cancer cells. *Free Radic. Biol. Med.* 50, 1771–1779. doi: 10.1016/j.freeradbiomed.2011.03.013

Fiorentino, M., Grandaliano, G., Gesualdo, L., and Castellano, G. (2018). Acute kidney injury to chronic kidney disease transition. *Contrib. Nephrol.* 193, 45–54. doi: 10.1159/000484962

Fujita, H., Fujishima, H., Chida, S., Takahashi, K., Qi, Z., Kanetsuna, Y., et al. (2009). Reduction of renal superoxide dismutase in progressive diabetic nephropathy. *J. Am. Soc. Nephrol.* 20, 1303–1313. doi: 10.1681/asn.2008080844

Gansevoort, R. T., Correa-Rotter, R., Hemmelgarn, B. R., Jafar, T. H., and Heerspink, H. J. (2013). Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet* 382, 339–352.

Gao, Y. H., Li, C. X., Shen, S. M., Li, H., Chen, G. Q., Wei, Q., et al. (2013). Hypoxia-inducible factor 1α mediates the down-regulation of superoxide dismutase 2 in von Hippel-Lindau deficient renal clear cell carcinoma. *Biochem. Biophys. Res. Commun.* 435, 46–51. doi: 10.1016/j.bbrc.2013.04.034

Go, A. S., Chertow, G. M., Fan, D., McCulloch, C. E., and Hsu, C. Y. (2004). Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med.* 351, 1296–1305. doi: 10.1056/nejmoa041031

Gomes, P., Simão, S., Silva, E., Pinto, V., Amaral, J. S., Afonso, J., et al. (2009). Aging increases oxidative stress and renal expression of oxidant and antioxidant enzymes that are associated with an increased trend in systolic blood pressure. *Oxid. Med. Cell Longev.* 2, 138–145. doi: 10.4161/oxim.2.3.8819

Guarente, L. (2011). Franklin H. Epstein lecture: sirtuins, aging, and medicine. *N. Engl. J. Med.* 364, 2235–2244. doi: 10.1056/nejmra1100831

Guo, W., Adachi, T., Matsui, R., Xu, S., Jiang, B., Zou, M. H., et al. (2003). Quantitative assessment of tyrosine nitration of manganese superoxide dismutase in angiotensin II-infused rat kidney. *Am. J. Physiol. Heart Circ. Physiol.* 285, H1396–H1403.

Han, Y., Xu, X., Tang, C., Gao, P., Chen, X., Xiong, X., et al. (2018). Reactive oxygen species promote tubular injury in diabetic nephropathy: the role of the mitochondrial ros-txnip-nlrp3 biological axis. *Redox Biol.* 2018, 32–46. doi: 10.1016/j.redox.2018.02.013

He, L., Wei, Q., Liu, J., Yi, M., Liu, Y., Liu, H., et al. (2017). AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms. *Kidney Int.* 92, 1071–1083. doi: 10.1016/j.kint.2017.06.030

Holley, A. K., Dhar, S. K., Xu, Y., and St. Clair, D. K. (2012). Manganese superoxide dismutase: beyond life and death. *Amino Acids* 42, 139–158. doi: 10.1007/s00726-010-0600-9

Homsi, E., Mota da Silva, S. Jr., Machado de Brito, S., Bouçada Inácio Peixoto, E., Butori Lopes de Faria, J., and Janino, P. (2011). p53-Mediated oxidative stress and tubular injury in rats with glycerol-induced acute kidney injury. *Am. J. Nephrol.* 33, 49–59. doi: 10.1159/000322836

Hopper, R. K., Carroll, S., Aponte, A. M., Johnson, D. T., French, S., Shen, R. F., et al. (2006). Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry* 45, 2524–2536. doi: 10.1021/bi052475e

Hou, C. L., Wang, M. J., Sun, C., Huang, Y., Jin, S., Mu, X. P., et al. (2016). Protective effects of hydrogen sulfide in the ageing kidney. *Oxid. Med. Cell Longev.* 2016:7570489.

Hou, Y., Shi, Y., Han, B., Liu, X., Qiao, X., Qi, Y., et al. (2018). The antioxidant peptide SS31 prevents oxidative stress, downregulates CD36 and improves renal function in diabetic nephropathy. *Nephrol. Dial. Transplant.* 33, 1908–1918. doi: 10.1093/ndt/gfy021

Houldsworth, A., Hodgkinson, A., Shaw, S., Millward, A., and Demaine, A. G. (2015). Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic complications. *Gene* 569, 41–45. doi: 10.1016/j.gene.2015.04.006

Hussain, S. P., Amstad, P., He, P., Robles, A., Lupold, S., Kaneko, I., et al. (2004). p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res.* 64, 2350–2356. doi: 10.1158/0008-5472.can-2287-2

Imlay, J. A. (2003). Pathways of oxidative damage. *Annu. Rev. Microbiol.* 57, 395–418. doi: 10.1146/annurev.micro.57.030502.090938

Ishimoto, Y., Inagi, R., Yoshihara, D., Kugita, M., Nagao, S., Shimizu, A., et al. (2017). Mitochondrial abnormality facilitates cyst formation in autosomal dominant polycystic kidney disease. *Mol. Cell. Biol.* 7:e00337-17. doi: 10.1128/MCB.00337-17

Jacobs, K. M., Pennington, J. D., Bisht, K. S., Aykin-Burns, N., Kim, H. S., Mishra, M., et al. (2008). SIRT3 interacts with the daf-16 homolog FOXO3a in the mitochondria, as well as increases FOXO3a dependent gene expression. *Int. J. Biol. Sci.* 4, 291–299. doi: 10.7150/ijbs.4.291

Jankauskas, S. S., Plotnikov, E. Y., Morosanova, M. A., Pevzner, I. B., Zorova, L. D., Skulachev, V. P., et al. (2012). Mitochondria-targeted antioxidant SkQR1 ameliorates gentamycin-induced renal failure and hearing loss. *Biochemistry* 77, 666–670. doi: 10.1134/s0006297912060144

Jia, Z., Zhuang, Y., Hu, C., Zhang, X., Ding, G., Zhang, Y., et al. (2016). Albuminuria enhances NHE3 and NCC via stimulation of mitochondrial oxidative stress/angiotensin II axis. *Oncotarget* 7, 47134–47144. doi: 10.18632/oncotarget.9972

Jin, C., Qin, L., Shi, Y., Candas, D., Fan, M., Lu, C. L., et al. (2015). CDK4-mediated MnSOD activation and mitochondrial homeostasis in radioadaptive protection. *Free Radic. Biol. Med.* 81, 77–87. doi: 10.1016/j.freeradbiomed.2014.12.026

John, J. P., Pollak, A., and Lubec, G. (2009). Complete sequencing and oxidative modification of manganese superoxide dismutase in medulloblastoma cells. *Electrophoresis* 30, 3006–3016. doi: 10.1002/elps.200900168

Jones, P. L., Ping, D., and Boss, J. M. (1997). Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. *Mol. Cell. Biol.* 17, 6970–6981. doi: 10.1128/mcb.17.12.6970

Karnati, S., Luers, G., Pfreimer, S., and Baumgart-Vogt, E. (2013). Mammalian SOD2 is exclusively located in mitochondria and not present in peroxisomes. *Histochem. Cell Biol.* 140, 105–117. doi: 10.1007/s00418-013-1099-4

Kato, M., Yuan, H., Xu, Z. G., Lanting, L., Li, S. L., Wang, M., et al. (2006). Role of the Akt/FoxO3a pathway in TGF-beta1-mediated mesangial cell dysfunction: a novel mechanism related to diabetic kidney disease. *J. Am. Soc. Nephrol.* 17, 3325–3335. doi: 10.1681/asn.2006070754

Kawamata, H., and Manfredi, G. (2010). Import, maturation, and function of SOD1 and its copper chaperone CCS in the mitochondrial intermembrane space. *Antioxid. Redox Signal.* 13, 1375–1384. doi: 10.1089/ars.2010.3212

KDIGO (2012). KDIGO clinical practice guideline for acute kidney injury. *Kidney Int. Suppl.* 2, 1–138.

Kezic, A., Spasojevic, I., Lezaic, V., and Bajcetic, M. (2016). Mitochondria-targeted antioxidants: future perspectives in kidney ischemia reperfusion injury. *Oxid. Med. Cell Longev.* 2016:2950503. doi: 10.1155/2016/2950503

Kim, H. P., Roe, J. H., Chock, P. B., and Yim, M. B. (1999). Transcriptional activation of the human manganese superoxide dismutase gene mediated by tetradecanoylphorbol acetate. *J. Biol. Chem.* 274, 37455–37460. doi: 10.1074/jbc.274.52.37455

Kim, Y. S., Gupta Vallur, P., Phaëton, R., Mythreye, K., and Hempel, N. (2017). Insights into the dichotomous regulation of SOD2 in cancer. *Antioxidants* 6:E86. doi: 10.3390/antiox6040086

Kimura, T., Shiizaki, K., Akimoto, T., Shinzato, T., Shimizu, T., Kurosawa, A., et al. (2018). The impact of preserved Klotho gene expression on antioxidative stress activity in healthy kidney. *Am. J. Physiol. Renal Physiol.* 315, F345–F352.

Kitada, M., Kume, S., Imaizumi, N., and Koya, D. (2011). Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway. *Diabetes Metab. Res. Rev.* 60, 634–643. doi: 10.2337/db10-0386

Kitada, M., Ogura, Y., Monno, I., and Koya, D. (2019). Sirtuins and Type 2 diabetes: role in inflammation, oxidative stress, and mitochondrial function. *Front. Endocrinol.* 10:187. doi: 10.3389/fendo.2019.00187

Kops, G. J., Dansen, T. B., Polderman, P. E., Saarloos, I., Wirtz, K. W., Coffer, P. J., et al. (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419, 316–321. doi: 10.1038/nature01036

Kume, S., Uzu, T., Horiike, K., Chin-Kanasaki, M., Isshiki, K., Araki, S., et al. (2010). Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J. Clin. Invest.* 120, 1043–1055. doi: 10.1172/jci41376

Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., et al. (1997). Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 390, 45–51. doi: 10.1038/36285

Le Clef, N., Verhulst, A., D'Haese, P. C., and Vervaet, B. A. (2016). Unilateral renal ischemia-reperfusion as a robust model for acute to chronic kidney injury in mice. *PLoS One* 11:e0152153. doi: 10.1371/journal.pone.0152153

Lebovitz, R. M., Zhang, H., Vogel, H., Cartwright, J., Dionne, L., Lu, N., et al. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9782–9787. doi: 10.1073/pnas.93.18.9782

Lee, S. J., Choi, M. G., Kim, D. S., and Kim, T. W. (2006). Manganese superoxide dismutase gene polymorphism (V16A) is associated with stages of albuminuria in Korean type 2 diabetic patients. *Metabolism* 55, 1–7. doi: 10.1016/j.metabol.2005.04.030

Li, A., Zhang, X., Shu, M., Wu, M., Wang, J., Zhang, J., et al. (2017). Arctigenin suppresses renal interstitial fibrosis in a rat model of obstructive nephropathy. *Phytomedicine* 30, 28–41. doi: 10.1016/j.phymed.2017.03.003

Li, C., Matavelli, L. C., Akhtar, S., and Siragy, H. M. (2019). Prorenin receptor contributes to renal mitochondria dysfunction, apoptosis and fibrosis in diabetic mice. *Sci. Rep.* 9:11667. doi: 10.1038/s41598-019-47055-1

Li, M., Chiu, J. F., Mossman, B. T., and Fukagawa, N. K. (2006). Down-regulation of manganese-superoxide dismutase through phosphorylation of FOXO3a by Akt in explanted vascular smooth muscle cells from old rats. *J. Biol. Chem.* 281, 40429–40439. doi: 10.1074/jbc.m606596200

Li, T., He, S., Liu, S., Kong, Z., Wang, J., and Zhang, Y. (2015). Effects of different exercise durations on Keap1-Nrf2-ARE pathway activation in mouse skeletal muscle. *Free Radic. Res.* 49, 1269–1274. doi: 10.3109/10715762.2015.1066784

Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., et al. (1995). Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376–381. doi: 10.1038/ng1295-376

Liang, H. L., Hilton, G., Mortensen, J., Regner, K., Johnson, C. P., and Nilakantan, V. (2009). MnTMPyP, a cell-permeant SOD mimetic, reduces oxidative stress and apoptosis following renal ischemia-reperfusion. *Am. J. Physiol. Renal Physiol.* 296, F266–F276.

Lim, J. H., Kim, E. N., Kim, M. Y., Chung, S., Shin, S. J., Kim, H. W., et al. (2012). Age-associated molecular changes in the kidney in aged mice. *Oxid. Med. Cell Longev.* 2012:171383. doi: 10.1155/2012/171383

Lin, J., Handschin, C., and Spiegelman, B. M. (2005). Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab.* 1, 361–370. doi: 10.1016/j.cmet.2005.05.004

Liu, L., Zheng, T., Wang, N., Wang, F., Li, M., Jiang, J., et al. (2009). The manganese superoxide dismutase Val16Ala polymorphism is associated with decreased risk of diabetic nephropathy in Chinese patients with type 2 diabetes. *Mol. Cell. Biochem.* 322, 87–91. doi: 10.1007/s11010-008-9943-x

Liu, M., Zhu, Y., Sun, Y., Wen, Z., Huang, S., Ding, G., et al. (2017). MnTBAP therapy attenuates the downregulation of sodium transporters in obstructive kidney disease. *Oncotarget* 9, 394–403. doi: 10.18632/oncotarget.23037

Lowes, D. A., Thottakam, B. M., Webster, N. R., Murphy, M. P., and Galley, H. F. (2008). The mitochondria-targeted antioxidant MitoQ protects against organ damage in a lipopolysaccharide-peptidoglycan model of sepsis. *Free Radic. Biol. Med.* 45, 1559–1565. doi: 10.1016/j.freeradbiomed.2008.09.003

Lu, Q., Zhai, Y., Cheng, Q., Liu, Y., Gao, X., Zhang, T., et al. (2013). The Akt-FoxO3a-manganese superoxide dismutase pathway is involved in the regulation of oxidative stress in diabetic nephropathy. *Exp. Physiol.* 98, 934–945. doi: 10.1113/expphysiol.2012.068361

Lu, Z., Xu, X., Hu, X., Fassett, J., Zhu, G., Tao, Y., et al. (2010). PGC-1 alpha regulates expression of myocardial mitochondrial antioxidants and myocardial oxidative stress after chronic systolic overload. *Antioxid. Redox. Signal.* 13, 1011–1022. doi: 10.1089/ars.2009.2940

MacMillan-Crow, L. A., Crow, J. P., Kerby, J. D., Beckman, J. S., and Thompson, J. A. (1996). Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc. Natl. Acad. Sci. U.S.A.* 93, 11853–11858. doi: 10.1073/pnas.93.21.11853

MacMillan-Crow, L. A., Crow, J. P., and Thompson, J. A. (1998). Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry* 37, 1613–1622. doi: 10.1021/bi971894b

MacMillan-Crow, L. A., Cruthirds, D. L., Ahki, K. M., Sanders, P. W., and Thompson, J. A. (2001). Mitochondrial tyrosine nitration precedes chronic allograft nephropathy. *Free Radic Biol. Med.* 31, 1603–1608. doi: 10.1016/s0891-5849(01)00750-x

Mailloux, R. J. (2015). Teaching the fundamentals of electron transfer reactions in mitochondria and the production and detection of reactive oxygen species. *Redox Biol.* 4, 381–398. doi: 10.1016/j.redox.2015.02.001

Mailloux, R. J., and Willmore, W. G. (2014). S-glutathionylation reactions in mitochondrial function and disease. *Front. Cell Dev. Biol.* 2:68. doi: 10.3389/fcell.2014.00068

Majzunova, M., Kvandova, M., Berenyiova, A., Balis, P., Dovinova, I., and Cacanyiova, S. (2019). Chronic NOS inhibition affects oxidative state and antioxidant response differently in the kidneys of young normotensive and hypertensive rats. *Oxid. Med. Cell Longev.* 2019:5349398. doi: 10.1155/2019/5349398

Miriyala, S., Spasojevic, I., Tovmasyan, A., Salvemini, D., Vujaskovic, Z., and St. Clair, D. (2012). Manganese superoxide dismutase, MnSOD and its mimics. *Biochim. Biophys. Acta* 1822, 794–814.

Möllsten, A., Marklund, S. L., Wessman, M., Svensson, M., Forsblom, C., Parkkonen, M., et al. (2007). A functional polymorphism in the manganese superoxide dismutase gene and diabetic nephropathy. *Diabetes Metab. Res. Rev.* 56, 265–269. doi: 10.2337/db06-0698

Morigi, M., Perico, L., Corna, D., Locatelli, M., Cassis, P., Carminati, C. E., et al. (2020). C3a receptor blockade protects podocytes from injury in diabetic nephropathy. *JCI Insight.* 5:131849. doi: 10.1172/jci.insight.131849

Mukhopadhyay, P., Horvath, B., Zsengeller, Z., Zielonka, J., Tanchian, G., Holovac, E., et al. (2012). Mitochondrial-targeted antioxidants represent a promising approach for prevention of cisplatin-induced nephropathy. *Free Radic. Biol. Med.* 52, 497–506. doi: 10.1016/j.freeradbiomed.2011.11.001

Murtas, C., Bruschi, M., Candiano, G., Moroni, G., Magistroni, R., Magnano, A., et al. (2012). Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy. *Clin. J. Am. Soc. Nephrol.* 7, 1394–1400. doi: 10.2215/cjn.02170312

Narita, T., Weinert, B. T., and Choudhary, C. (2019). Functions and mechanisms of non-histone protein acetylation. *Nat. Rev. Mol. Cell Biol.* 20, 156–174. doi: 10.1038/s41580-018-0081-3

Ninomiya, T., Perkovic, V., de Galan, B. E., Zoungas, S., Pillai, A., Jardine, M., et al. (2009). Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. *J. Am. Soc. Nephrol.* 20, 1813–1821. doi: 10.1681/asn.2008121270

Noiri, E., Nakao, A., Uchida, K., Tsukahara, H., Ohno, M., Fujita, T., et al. (2001). Oxidative and nitrosative stress in acute renal ischemia. *Am. J. Physiol. Renal Physiol.* 281, F948–F957.

Nomiyama, T., Tanaka, Y., Piao, L., Nagasaka, K., Sakai, K., Ogihara, T., et al. (2003). The polymorphism of manganese superoxide dismutase is associated with diabetic nephropathy in Japanese type 2 diabetic patients. *J. Hum. Genet.* 48, 138–141.

Ogura, Y., Kitada, M., Monno, I., Kanasaki, K., Watanabe, A., and Koya, D. (2018). Renal mitochondrial oxidative stress is enhanced by the reduction of Sirt3 activity, in Zucker diabetic fatty rats. *Redox Rep.* 23, 153–159. doi: 10.1080/13510002.2018.1487174

Ogura, Y., Kitada, M., Xu, J., Monno, I., and Koya, D. (2020). CD38 inhibition by apigenin ameliorates mitochondrial oxidative stress through restoration of the intracellular NAD^+/NADH ratio and Sirt3 activity in renal tubular cells in diabetic rats. *Aging (Albany NY)* 12, 11325–11336. doi: 10.18632/aging.103410

Onozato, M. L., Tojo, A., Goto, A., and Fujita, T. (2004). Radical scavenging effect of gliclazide in diabetic rats fed with a high cholesterol diet. *Kidney Int.* 65, 951–960. doi: 10.1111/j.1523-1755.2004.00470.x

Onozato, M. L., Tojo, A., Goto, A., Fujita, T., and Wilcox, C. S. (2002). Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int.* 61, 186–194. doi: 10.1046/j.1523-1755.2002.00123.x

Ouyang, J., Zeng, Z., Fang, H., Li, F., Zhang, X., and Tan, W. (2019). SIRT3 inactivation promotes acute kidney injury through elevated acetylation of SOD2 and p53. *J. Surg. Res.* 233, 221–230. doi: 10.1016/j.jss.2018.07.019

Pani, G., Bedogni, B., Anzevino, R., Colavitti, R., Palazzotti, B., Borrello, S., et al. (2000). Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. *Cancer Res.* 60, 4654–4660.

Parajuli, N., Marine, A., Simmons, S., Saba, H., Mitchell, T., Shimizu, T., et al. (2011). Generation and characterization of a novel kidney-specific manganese superoxide dismutase knockout mouse. *Free Radic. Biol. Med.* 51, 406–416. doi: 10.1016/j.freeradbiomed.2011.04.024

Patil, N. K., Parajuli, N., MacMillan-Crow, L. A., and Mayeux, P. R. (2014). Inactivation of renal mitochondrial respiratory complexes and manganese superoxide dismutase during sepsis: mitochondria-targeted antioxidant mitigates injury. *Am. J. Physiol. Renal Physiol.* 306, F734–F743.

Patil, N. K., Saba, H., and MacMillan-Crow, L. A. (2013). Effect of S-nitrosoglutathione on renal mitochondrial function: a new mechanism for reversible regulation of manganese superoxide dismutase activity? *Free Radic. Biol. Med.* 56, 54–63. doi: 10.1016/j.freeradbiomed.2012.12.001

Plotnikov, E. Y., Chupyrkina, A. A., Jankauskas, S. S., Pevzner, I. B., Silachev, D. N., Skulachev, V. P., et al. (2011). Mechanisms of nephroprotective effect of mitochondria-targeted antioxidants under rhabdomyolysis and ischemia/reperfusion. *Biochim. Biophys. Acta* 1812, 77–86. doi: 10.1016/j.bbadis.2010.09.008

Porteous, C. M., Logan, A., Evans, C., Ledgerwood, E. C., Menon, D. K., Aigbirhio, F., et al. (2010). Rapid uptake of lipophilic triphenylphosphonium cations by mitochondria in vivo following intravenous injection: implications for mitochondria-specific therapies and probes. *Biochim. Biophys. Acta* 1800, 1009–1017. doi: 10.1016/j.bbagen.2010.06.001

Prunotto, M., Carnevali, M. L., Candiano, G., Murtas, C., Bruschi, M., Corradini, E., et al. (2010). Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. *J. Am. Soc. Nephrol.* 21, 507–519. doi: 10.1681/asn.2008121259

Qiu, X., Brown, K., Hirschey, M. D., Verdin, E., and Chen, D. (2010). Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab.* 12, 662–667. doi: 10.1016/j.cmet.2010.11.015

Radi, R. (2018). Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc. Natl. Acad. Sci. U.S.A.* 115, 5839–5848. doi: 10.1073/pnas.1804932115

Rattanavich, R., Plagov, A., Kumar, D., Rai, P., Lederman, R., Salhan, D., et al. (2013). Deficit of p66ShcA restores redox-sensitive stress response program in cisplatin-induced acute kidney injury. *Exp. Mol. Pathol.* 94, 445–452. doi: 10.1016/j.yexmp.2013.03.001

Rodriguez-Iturbe, B., Sepassi, L., Quiroz, Y., Ni, Z., Wallace, D. C., and Vaziri, N. D. (2007). Association of mitochondrial SOD deficiency with salt-sensitive hypertension and accelerated renal senescence. *J. Appl. Physiol.* 102, 255–260. doi: 10.1152/japplphysiol.00513.2006

Schrier, R. W., and Wang, W. (2004). Acute renal failure and sepsis. *N. Engl. J. Med.* 351, 159–169.

Shabalina, I. G., Vyssokikh, M. Y., Gibanova, N., Csikasz, R. I., Edgar, D., Hallden-Waldemarson, A., et al. (2017). Improved health-span and lifespan in mtDNA mutator mice treated with the mitochondrially targeted antioxidant SkQ1. *Aging* 9, 315–339. doi: 10.18632/aging.101174

Sharma, K. (2017). Mitochondrial dysfunction in the diabetic kidney. *Adv. Exp. Med. Biol.* 982, 553–562. doi: 10.1007/978-3-319-55330-6_28

Shimoda-Matsubayashi, S., Matsumine, H., Kobayashi, T., Nakagawa-Hattori, Y., Shimizu, Y., and Mizuno, Y. (1996). Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem. Biophys. Res. Commun.* 226, 561–565. doi: 10.1006/bbrc.1996.1394

Shrestha, B., and Haylor, J. (2016). Evolution of the concept and pathogenesis of chronic renal allograft injury: an updated review. *Exp. Clin. Transplant.* 14, 596–605.

Smith, R. A., and Murphy, M. P. (2010). Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Ann. N. Y. Acad. Sci.* 1201, 96–103. doi: 10.1111/j.1749-6632.2010.05627.x

Smith, R. A. J., Porteous, C. M., Gane, A. M., and Murphy, M. P. (2003). Delivery of bioactive molecules to mitochondria in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 100, 5407–5412. doi: 10.1073/pnas.0931245100

Spencer, N. Y., Yang, Z., Sullivan, J. C., Klein, T., and Stanton, R. C. (2018). Linagliptin unmasks specific antioxidant pathways protective against albuminuria and kidney hypertrophy in a mouse model of diabetes. *PLoS One* 13:e0200249. doi: 10.1371/journal.pone.0200249

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jager, S., et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127, 397–408. doi: 10.1016/j.cell.2006.09.024

Sutton, A., Khoury, H., Prip-Buus, C., Cepanec, C., Pessayre, D., and Degoul, F. (2003). The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics* 13, 145–157.

Szeto, H. H., Liu, S., Soong, Y., Wu, D., Darrah, S. F., Cheng, F. Y., et al. (2011). Mitochondria-targeted peptide accelerates ATP recovery and reduces ischemic kidney injury. *J. Am. Soc. Nephrol.* 22, 1041–1052. doi: 10.1681/asn.2010080808

Tang, Z., Shou, I., Wang, L. N., Fukui, M., and Tomino, Y. (1997). Effects of antihypertensive drugs or glycemic control on antioxidant enzyme activities in spontaneously hypertensive rats with diabetes. *Nephron* 76, 323–330. doi: 10.1159/000190199

Tao, R., Coleman, M. C., Pennington, J. D., Ozden, O., Park, S. H., Jiang, H., et al. (2010). Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol. Cell.* 40, 893–904. doi: 10.1016/j.molcel.2010.12.013

Taylor, R. C., Acquaah-Mensah, G., Singhal, M., Malhotra, D., and Biswal, S. (2008). Network inference algorithms elucidate Nrf2 regulation of mouse lung oxidative stress. *PLoS Comput. Biol.* 4:e1000166. doi: 10.1371/journal.pcbi.1000166

Teissier, T., Quersin, V., Gnemmi, V., Daroux, M., Howsam, M., Delguste, F., et al. (2019). Knockout of receptor for advanced glycation end-products attenuates age-related renal lesions. *Aging Cell* 18, e12850. doi: 10.1111/acel.12850

Tharmalingam, S., Alhasawi, A., Appanna, V. P., Lemire, J., and Appanna, V. D. (2017). Reactive nitrogen species (RNS)-resistant microbes: adaptation and medical implications. *Biol. Chem.* 398, 1193–1208. doi: 10.1515/hsz-2017-0152

Thuraisingham, R. C., Nott, C. A., Dodd, S. M., and Yaqoob, M. M. (2000). Increased nitrotyrosine staining in kidneys from patients with diabetic nephropathy. *Kidney Int.* 57, 1968–1972. doi: 10.1046/j.1523-1755.2000.00046.x

Tian, C., Fang, S., Du, X., and Jia, C. (2011). Association of the C47T polymorphism in SOD2 with diabetes mellitus and diabetic microvascular complications: a meta-analysis. *Diabetologia* 54, 803–811. doi: 10.1007/s00125-010-2004-5

Valle, I., varez-Barrientos, A., Arza, E., Lamas, S., and Monsalve, M. (2005). PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc. Res.* 66, 562–573. doi: 10.1016/j.cardiores.2005.01.026

van de Ven, R. A. H., Santos, D., and Haigis, M. C. (2017). Mitochondrial sirtuins and molecular mechanisms of aging. *Trends Mol. Med.* 23, 320–331. doi: 10.1016/j.molmed.2017.02.005

Van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S. R., et al. (2003). Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genom.* 16, 29–37. doi: 10.1152/physiolgenomics.00122.2003

Vlassara, H., Torreggiani, M., Post, J. B., Zheng, F., Uribarri, J., and Striker, G. E. (2009). Role of oxidants/inflammation in declining renal function in chronic kidney disease and normal aging. *Kidney Int. Suppl.* 76, S3–S11.

Walker, L. M., Walker, P. D., Imam, S. Z., Ali, S. F., and Mayeux, P. R. (2000). Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N(6)-(1-Iminoethyl)lysine. *J. Pharmacol. Exp. Ther.* 295, 417–422.

Wang, H. D., Xu, S. Q., Johns, D. G., Du, Y., Quinn, M. T., Cayatte, A. J., et al. (2001). Role of NADPH oxidase in the vascular hypertrophic and oxidative stress response to angiotensin II in mice. *Circ. Res.* 88, 947–953. doi: 10.1161/hh0901.089987

Wang, Y., Branicky, R., Noë, A., and Hekimi, S. (2018). Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. *J. Cell Biol.* 217, 1915–1928. doi: 10.1083/jcb.201708007

Warner, B. B., Stuart, L., Gebb, S., and Wispé, J. R. (1996). Redox regulation of manganese superoxide dismutase. *Am. J. Physiol.* 271, L150–L158.

Williams, M. D., Van Remmen, H., Conrad, C. C., Huang, T. T., Epstein, C. J., and Richardson, A. (1998). Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J. Biol. Chem.* 273, 28510–28515. doi: 10.1074/jbc.273.43.28510

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98, 115–124. doi: 10.1016/s0092-8674(00)80611-x

Xiao, L., Xu, X., Zhang, F., Wang, M., Xu, Y., Tang, D., et al. (2017). The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox. Biol.* 11, 297–311. doi: 10.1016/j.redox.2016.12.022

Xu, J., Kitada, M., and Koya, D. (2020). The impact of mitochondrial quality control by Sirtuins on the treatment of type 2 diabetes and diabetic kidney disease. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866:165756. doi: 10.1016/j.bbadis.2020.165756

Xu, S. (2016). SIRT1/3 activation by resveratrol attenuates acute kidney injury in a septic rat model. *Oxid. Med. Cell Longev.* 2016:7296092. doi: 10.1155/2016/7296092

Xu, S., Jiang, B., Maitland, K. A., Bayat, H., Gu, J., Nadler, J. L., et al. (2006). The thromboxane receptor antagonist S18886 attenuates renal oxidant stress and proteinuria in diabetic apolipoprotein E-deficient mice. *Diabetes Metab. Res. Rev.* 55, 110–119. doi: 10.2337/diabetes.55.01.06.db05-0831

Xu, Y., Kiningham, K. K., Devalaraja, M. N., Yeh, C. C., Majima, H., Kasarskis, E. J., et al. (1999). An intronic NF-kappaB element is essential for induction of the human manganese superoxide dismutase gene by tumor necrosis factor-alpha and interleukin-1beta. *DNA Cell Biol.* 18, 709–722. doi: 10.1089/104454999314999

Xu, Y., Porntadavity, S., and St Clair, D. K. (2002). Transcriptional regulation of the human manganese superoxide dismutase gene: the role of specificity protein 1 (Sp1) and activating protein-2 (AP-2). *Biochem. J.* 362, 401–412. doi: 10.1042/bj3620401

Yamakura, F., and Kawasaki, H. (2010). Post-translational modifications of superoxide dismutase. *Biochim. Biophys. Acta* 1804, 318–325. doi: 10.1016/j.bbapap.2009.10.010

Yamakura, F., Taka, H., Fujimura, T., and Murayama, K. (1998). Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *J. Biol. Chem.* 273, 14085–14089. doi: 10.1074/jbc.273.23.14085

Yamamoto, M., Clark, J. D., Pastor, J. V., Gurnani, P., Nandi, A., Kurosu, H., et al. (2005). Regulation of oxidative stress by the anti-aging hormone klotho. *J. Biol. Chem.* 280, 38029–38034. doi: 10.1074/jbc.m509039200

Yi, X., Nickeleit, V., James, L. R., and Maeda, N. (2011). α-Lipoic acid protects diabetic apolipoprotein E-deficient mice from nephropathy. *J. Diabetes Complicat.* 25, 193–201. doi: 10.1016/j.jdiacomp.2010.07.004

Zayed, A. E., Saleh, A., Gomaa, A. M. S., Abd-Elkareem, M., and Anwar, M. M. (2018). Protective effect of ginkgo biloba and magnetized water on nephropathy in induced type 2 diabetes in rat. *Oxid. Med. Cell Longev.* 2018:1785614. doi: 10.1155/2018/1785614

Zhang, W., Sha, Y., Wei, K., Wu, C., Ding, D., Yang, Y., et al. (2018). Rotenone ameliorates chronic renal injury caused by acute ischemia/reperfusion. *Oncotarget* 9, 24199–24208. doi: 10.18632/oncotarget.24733

Zhang, W., Yang, Y., Gao, H., Zhang, Y., Jia, Z., and Huang, S. (2019). Inhibition of mitochondrial complex i aggravates folic acid-induced acute kidney injury. *Kidney Blood Press Res.* 44, 1002–1013. doi: 10.1159/000501934

Zhao, Z., Azadzoi, K. M., Choi, H. P., Jing, R., Lu, X., Li, C., et al. (2017). LC-MS/MS analysis unravels deep oxidation of manganese superoxide dismutase in kidney cancer. *Int. J. Mol. Sci.* 18:E319. doi: 10.3390/ijms18020319

Zhu, C. H., Huang, Y., Oberley, L. W., and Domann, F. E. (2001). A family of AP-2 proteins down-regulate manganese superoxide dismutase expression. *J. Biol. Chem.* 276, 14407–14413. doi: 10.1074/jbc.m009708200

Zhuang, Y., Yasinta, M., Hu, C., Zhao, M., Ding, G., Bai, M., et al. (2015). Mitochondrial dysfunction confers albumin-induced NLRP3 inflammasome activation and renal tubular injury. *Am. J. Physiol. Renal Physiol.* 308, F857–F866.

Conflict of Interest: Boehringer Ingelheim, Mitsubishi Tanabe Pharma, Kyowa Kirin, Taisho Pharmaceutical Co., and Ono Pharmaceutical Co., contributed to establishing the Division of Anticipatory Molecular Food Science and Technology.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cell and Developmental Biology

REVIEW
published: 02 September 2020
doi: 10.3389/fcell.2020.00848

Iron and Cadmium Entry Into Renal Mitochondria: Physiological and Toxicological Implications

Frank Thévenod[1], Wing-Kee Lee[1] and Michael D. Garrick[2]*

[1] Faculty of Health, Centre for Biomedical Education and Research, Institute of Physiology, Pathophysiology and Toxicology, Witten/Herdecke University, Witten, Germany, [2] Department of Biochemistry, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, United States

Regulation of body fluid homeostasis is a major renal function, occurring largely through epithelial solute transport in various nephron segments driven by Na^+/K^+-ATPase activity. Energy demands are greatest in the proximal tubule and thick ascending limb where mitochondrial ATP production occurs through oxidative phosphorylation. Mitochondria contain 20–80% of the cell's iron, copper, and manganese that are imported for their redox properties, primarily for electron transport. Redox reactions, however, also lead to reactive, toxic compounds, hence careful control of redox-active metal import into mitochondria is necessary. Current dogma claims the outer mitochondrial membrane (OMM) is freely permeable to metal ions, while the inner mitochondrial membrane (IMM) is selectively permeable. Yet we recently showed iron and manganese import at the OMM involves divalent metal transporter 1 (DMT1), an H^+-coupled metal ion transporter. Thus, iron import is not only regulated by IMM mitoferrins, but also depends on the OMM to intermembrane space H^+ gradient. We discuss how these mitochondrial transport processes contribute to renal injury in systemic (e.g., hemochromatosis) and local (e.g., hemoglobinuria) iron overload. Furthermore, the environmental toxicant cadmium selectively damages kidney mitochondria by "ionic mimicry" utilizing iron and calcium transporters, such as OMM DMT1 or IMM calcium uniporter, and by disrupting the electron transport chain. Consequently, unraveling mitochondrial metal ion transport may help develop new strategies to prevent kidney injury induced by metals.

Keywords: reactive oxygen species, divalent metal transporter 1, ionic mimicry, manganese, copper, nephrotoxicity, acute kidney injury, chronic kidney disease

OPEN ACCESS

Edited by:
Giuliano Ciarimboli, University of Münster, Germany

Reviewed by:
Rosalinde Masereeuw, Utrecht University, Netherlands
Carlos Palmeira, University of Coimbra, Portugal

****Correspondence:***
Frank Thévenod
frank.thevenod@uni-wh.de

Specialty section:
This article was submitted to Mitochondrial Research, a section of the journal Frontiers in Cell and Developmental Biology

Received: *04 June 2020*
Accepted: *07 August 2020*
Published: *02 September 2020*

Citation:
Thévenod F, Lee W-K and Garrick MD (2020) Iron and Cadmium Entry Into Renal Mitochondria: Physiological and Toxicological Implications. Front. Cell Dev. Biol. 8:848. doi: 10.3389/fcell.2020.00848

INTRODUCTION

Together with the lungs and intestines, the kidneys maintain mammalian body fluid homeostasis by excreting most metabolic end-products and controlling the concentration of most constituents. Details of kidney morphology, structure of the functional unit, the nephron, and physiology are found in handbooks of renal physiology (Windhager, 1992; Brenner, 2008; Alpern et al., 2013). To prevent critical losses and ensuing deficiencies, nephrons, which form a tubular system,

actively reabsorb essential nutrients and electrolytes from primary fluid as well as actively secrete metabolic wastes since the rate of waste production exceeds their rate of glomerular filtration. Transport of solutes and water across tubule membranes occurs via passive transport (diffusive flux through channels and carrier-mediated facilitated diffusion) and active transport (primary active pumps/ATPases, secondary or tertiary active electrochemical potential-driven transporters). Active transport requires energy from adenosine triphosphate (ATP) for activation of basolateral Na^+/K^+-ATPases to maintain active reabsorption and secretion processes.

NEPHRON TRANSPORT AND METABOLISM: FOCUSING ON ENERGY

Among other functions, relevant to this review is the role of the proximal tubule (PT) in taking up filtered proteins and peptides via an apical multi-ligand receptor complex, megalin:cubilin:amnionless (Christensen and Birn, 2002), including metalloproteins, e.g., transferrin (Tf), an iron [Fe (III)] binding protein, and metallothionein (MT), a cadmium [Cd (II)] binding protein. The loop of Henle (LOH) establishes the medullary hyperosmotic interstitium that is required for reabsorption of water in the collecting duct (CD), to generate small volumes of concentrated urine ("antidiuresis"), thus preserving water for the body. From the viewpoint of energetics, the "motor" of the concentration gradient in the medulla is active NaCl reabsorption into the interstitium in the thick ascending limb of the LOH (via apical $Na^+/K^+/2Cl^-$ cotransport mediated by SLC12A1, basolateral Na^+/K^+-ATPase and Cl^- channel ClC-Kb/K2), and resulting in hypo-osmotic tubular fluid entering the distal tubule (DT) (Windhager, 1992; Brenner, 2008; Alpern et al., 2013). The DT reabsorbs divalent metal ions from the hypo-osmotic fluid, including Fe (II) via the divalent metal transporter (DMT1/SLC11A2), and the luminal fluid is further ion depleted by active NaCl symport via SLC12A3. Finally, the CD fine-tunes urine composition through hormonal regulation of principal (light) CD cells via aldosterone and antidiuretic hormone by transient, regulated incorporation of epithelial Na^+ (ENaC) and aquaporin-2 (AQP2) water channels, respectively, into the apical membrane.

To drive these transport processes, Na^+/K^+-ATPase is highly expressed in the basolateral membrane of renal tubule cells (Katz, 1982). The Na^+/K^+-ATPase activity profile along the nephron shows highest activity in the thick ascending limb of the LOH and DT, followed by the PT, whereas Na^+/K^+-ATPase is present in lesser amounts in other segments (Katz et al., 1979). These differences are paralleled by surface area distribution of the basolateral membrane (e.g., via infolded plasma membranes), the mitochondrial density, and IMM surface (Pfaller, 1982; reviewed in Guder and Ross, 1984), indicating a functional relation between ATP-forming and ATP-using structures.

Two relevant pathways of ATP production are glycolysis versus mitochondrial respiration via fatty acid oxidation (FAO) plus oxidative phosphorylation (OXPHOS), with the latter predominant in the PT and LOH whereas the former is more prominent in the distal nephron. All nephron segments except the PT, utilize glycolysis. Glycolysis can be anaerobic or aerobic. With oxygen, cytosolic glycolysis drives shuttling of pyruvate across the IMM, to consumption in the tricarboxylic acid (TCA) cycle in the mitochondrial matrix (Mccommis and Finck, 2015). The TCA cycle does not directly produce much ATP, but NADH and $FADH_2$ feed electrons into the electron transport chain (ETC) (see section "Electron Transport Chain") prior to OXPHOS to drive ATP production (Fernie et al., 2004). In contrast, gluconeogenesis predominantly takes place in PT and CD (reviewed in Schmidt and Guder, 1976; Guder and Ross, 1984; Wirthensohn and Guder, 1986). Notably, acute kidney injury (AKI) induced by hypoxia or ischemia displays impaired energetics in highly metabolically active nephron segments, such as PT and thick ascending LOH, and is associated with mitochondrial dysfunction and ATP depletion (Basile et al., 2012) (see sections "Fe Overload and Renal Injury" and "Fe and Mitochondrial Damage"). Hence, mitochondria are of crucial importance for renal function by supplying ATP that is essential for Na^+/K^+-ATPase activity.

MITOCHONDRIAL FUNCTIONS: DETERMINANTS OF LIFE AND DEATH

Electron Transport Chain

The ETC comprises five multimeric complexes (CI–CV) localized in the IMM. Electrons are shuttled from the multivalent metal core of one complex to the next via redox reactions, aided by ubiquinone and cytochrome c (cytC) on either side of CIII, and generating energy for transfer of protons from the matrix to the IMS. The electrons ultimately transfer to the final electron acceptor, oxygen, to form H_2O (Letts and Sazanov, 2017). Protons flow back through complex V, the F_1-F_0 ATP synthase, using the energy stored in the two components of the matrix-directed proton-motive force, a pH differential and an electrical membrane potential ($\Delta\psi m$), a process called chemiosmosis, to form ATP. Release of the stored energy initiates two rotary motors: the ring of c subunits in F_0 (around subunit a), along with subunits γ, δ and ε in F_1, to which F_0 is attached. Protons pass F_0 via subunit a to the c-ring (Wittig and Schagger, 2008). Rotation of subunit γ within the F_1 $\alpha 3\beta 3$ hexamer provides energy for ATP synthesis. This process is called "rotary catalysis" (reviewed in Jonckheere et al., 2012). During proton translocation, a steep potential gradient is generated that exerts a force on a deprotonated glutamate, resulting in net directional rotation, converted into chemical energy (Klusch et al., 2017). This energy is stored in phospho-anhydride bonds in the ATP molecule, and then liberated in exergonic hydrolysis reactions by cellular ATPases to power energy-dependent cellular processes. A lateral chemical H^+ gradient from complex IV of the ETC (lower local pH) to F_1-F_0 ATP synthase (higher local pH) resulting from the proton sink generated by proton transport through the F_1-F_0 ATP synthase may necessitate a modification to Peter Mitchell's chemiosmotic model (Rieger et al., 2014). Further, some electrons "leak" back through the membrane as byproducts of single electron escape from ETC complexes, OXPHOS and matrix

biochemical reactions, resulting in the production of noxious reactive oxygen species (ROS), usually superoxide anion ($O_2\bullet^-$) or hydrogen peroxide (H_2O_2) (Munro and Treberg, 2017), which can be detoxified (see section "What to Do About/With So Many Radicals?").

Mitochondrial ROS Generation

Undoubtedly, the ETC is the major source of mitochondrial ROS (mtROS) (see section "Electron Transport Chain") (**Figure 1A**). Through their flavin- and quinone-binding sites, CI and CIII are the largest ETC ROS generators (I > III > II >> IV), mostly superoxide, that are deposited into the matrix (Yan et al., 2020). CI and CII generate matrix H_2O_2, and CIII's superoxide can also be found in the cristae lumen and IMS (Brand, 2016) where it traverses the OMM through the voltage dependent anion channel (VDAC) into the cytosol (Han et al., 2003) and can impact cellular signaling pathways following its conversion to H_2O_2.

Additional sources of mtROS stem from substrate metabolism and OXPHOS (Quinlan et al., 2014; Brand, 2016; Sies and Jones, 2020). In fact, the 2-oxoacid dehydrogenase complex (2-OADC) can generate approximately eight times the amount of ROS as CI (Quinlan et al., 2014)! Superoxide/H_2O_2 generated through these sources are deposited in the matrix. ROS can also be generated by more finely controlled activity of NADPH oxidases (NOX) located in cellular membranes and constitute a physiological signaling pathway (Bedard and Krause, 2007; Buvelot et al., 2019). NOX1–3 and NOX5 generate superoxide whereas NOX4 is constitutively active and generates H_2O_2 (Bedard and Krause, 2007; Nisimoto et al., 2014). NOX4 was localized to the IMM (Block et al., 2009) of renal PT HK-2 cells and was negatively regulated by ATP (Shanmugasundaram et al., 2017), suggesting OXPHOS activity uses ROS signals to regulate mitochondrial activity or signaling.

Notably, mitochondrial superoxide/H_2O_2 production can be modulated by both intra- and extramitochondrial cues, such as through proton leak back into the matrix, formation of super-complexes from one or more ETC complexes, or redox status (reviewed in Kuksal et al., 2017).

Apoptosis Signaling

Apoptotic stimuli, involving calcium or ROS, induce the IMM to undergo mitochondrial permeability transition by triggering formation of a mitochondrial permeability transition pore (mPTP) (see also section "Cd and Kidney Mitochondrial Damage") at contact sites between the IMM and OMM. The molecular composition of mPTP, once thought to contain OMM VDAC, IMM ANT, and matrix cyclophilin D, has remained elusive until now (reviewed in Baines and Gutierrez-Aguilar, 2018; Bonora and Pinton, 2019). Consequently, the IMM is no longer selective and there is a sudden increase in the permeability of the IMM to solutes up to 1500 Da, which also dissipates $\Delta\psi_m$. Uncontrolled flux of solutes and water into the matrix occurs, concluding with an increase in matrix volume due to osmotic pressure increase and matrix expansion. The IMM with its increased surface area disrupts the OMM culminating in the release of pro-apoptotic factors, like cytC or apoptosis inducing factor, from the IMS resulting in general mitochondrial dysfunction (Kroemer et al., 2007). Dissociation of cytC from cardiolipin is promoted by ROS resulting in its transfer from a tightly- to a loosely-bound pool (Petrosillo et al., 2003) [cytC is tightly bound to the IMM by electrostatic interactions (Ott et al., 2002)], triggering apoptosis (Brown and Borutaite, 2008).

METAL DISTRIBUTION IN MITOCHONDRIA – TO WHAT PURPOSE?

An early study on ^{56}manganese (Mn) flux in rats noted that metal uptake was enriched in liver, pancreas and kidney and found ∼40% of liver and ∼64% of renal uptake in mitochondria (Maynard and Cotzias, 1955; Sakurai et al., 1985). In the last decade, development of biophysical methods allows estimation not only of organelle Fe concentration *in situ* but also speciation (Dlouhy and Outten, 2013). Application to yeast (Holmes-Hampton et al., 2013) yielded quantitative results where the authors stated "cells contained ∼150 μM Fe, distributed primarily into . . . mitochondrial Fe." Unfortunately, Lindahl's group has not yet provided such analyses for renal cells. Mitochondria in human bronchial epithelial cells contain ∼50% of total Fe content (Ghio et al., 2013; Ghio et al., 2016). In yeast, published data (Pierrel et al., 2007) suggest that 20% of cellular copper (Cu) resides in mitochondria. The relative richness of the mitochondrial endowment with these transition metals is consistent with the high proportion of redox functions that remain encoded in the small circular DNA of mitochondria while apparently many other organellar functions migrated to nuclear DNA.

The redox ability of Fe, Cu, and Mn to transition from one to another species accounts for their unusual utility; redox properties can also generate free radicals leading mostly to toxic effects for the same group of metals. For Fe, the electron donor is Fe^{2+}; the acceptor, Fe^{3+}. Many of the roles dependent on this property have been recently reviewed (Dlouhy and Outten, 2013). Remarkably many of the processes take place largely in mitochondria. Heme, as a cofactor, begins and ends its synthesis there and participates via cytochromes in the ETC. Biosynthesis of Fe-sulfur cluster proteins also involves mitochondria and cytosol (Lill et al., 2012) and also results in multiple proteins that function in the organelle (Dlouhy and Outten, 2013). While non-heme Fe also participates in multiple proteins, sorting out which one has mitochondrial effects is challenging for most of them. Nevertheless, one critical role for Fe is participating in the reduction of ribo- to deoxyribonucleotides in mitochondria through nuclear gene *RRM2B* (Bourdon et al., 2007). The *Rrm2b*$^{-/-}$ mouse exhibited inactivated ribonucleotide reductase that appeared in all analyzed tissues, including renal.

A major function of Mn and Cu in mitochondria is their critical role in metal-dependent superoxide dismutases (SODs) to detoxify superoxide (Zelko et al., 2002; Baker et al., 2017). Superoxide generation and SODs are considered below in sections "ROS/RNS: Double-Edged Swords" and "SODs". The IMS is the location where Cu-dependent cytC oxidase matures, with its function, denoted in its name, mitochondrial. Other

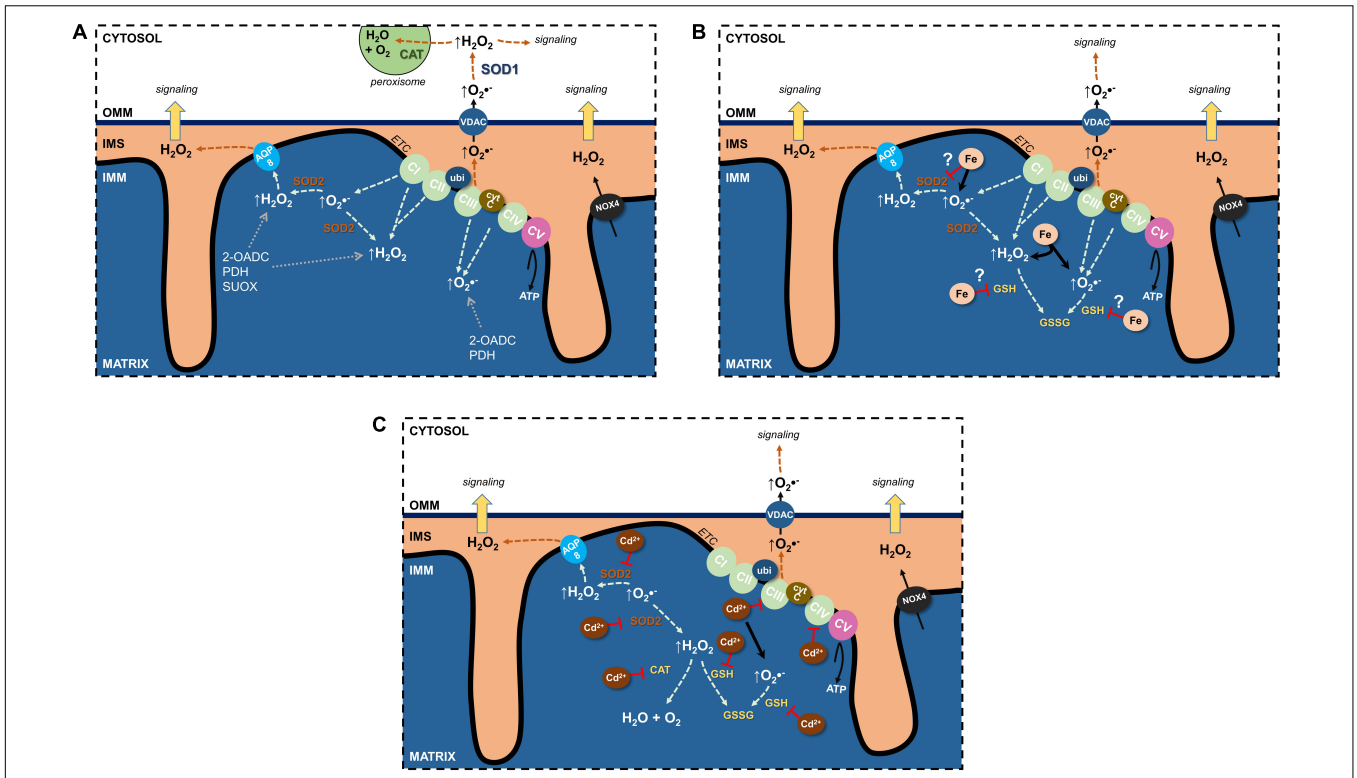


FIGURE 1 | Effects of Fe and Cd on mitochondrial reactive oxygen species (ROS). **(A)** Sources of physiological ROS from mitochondria and their release into the cytosol. **(B)** Fe increase mitochondrial ROS formation and potentially inhibits antioxidants. **(C)** Cd inhibits the electron transport chain, binds GSH as well as decreases antioxidant enzyme activity to augment mitochondrial ROS generation. See text for further details. 2-OADC, 2-oxoacid dehydrogenase complexes; AQP, aquaporin; CAT, catalase; cytc, cytochrome c; ETC, electron transport chain; GSH, glutathione; IMS, intermembrane space; NOX4, NADPH oxidase 4; PDH, pyruvate dehydrogenase; SOD, superoxide dismutase; SUOX, sulfite oxidase; ubi, ubiquinone; VDAC, voltage-dependent anion channel.

elements of Cu metabolism in the organelle were recently reviewed (Baker et al., 2017).

ROS/RNS: DOUBLE-EDGED SWORDS

Essential to mammalian life, molecular or diatomic oxygen (O_2) is a diradical with two unpaired electrons that participate in an unusual three-electron bond between each oxygen atom. The configuration of these unpaired electrons in each outer orbital shell makes O_2 particularly susceptible to free radical formation (Halliwell, 1991). The acceptance of an electron results in an anionic form of O_2, the superoxide anion $O_2\bullet^-$, highly reactive yet short-lived (Hayyan et al., 2016). Other free radicals include the hydroxyl radical (•OH) and (hydrogen) peroxide [$H_2(O_2\bullet^{-2})$]; henceforth, H_2O_2 (Munro and Treberg, 2017). Though H_2O_2 itself is not a free radical, •OH can be generated in the presence of redox metals (Fe, Cu) through the Fenton reaction. Moreover, superoxide can react with nitric oxide (NO•) to form peroxynitrite ($ONOO^-$) and NO• with CO_2 to yield nitrosoperoxycarbonate ($ONOOCO_2^-$). Similar to ROS, these reactive nitrogen species (RNS) are highly reactive and can oxidize thiols and nitrate proteins (Adams et al., 2015). Since RNS generation is directly dependent on superoxide (Valdez et al., 2018), focus will be placed on ROS.

ROS as Physiological Signals

Reactive oxygen species have long been regarded as unwanted and dangerous molecules that elicit cellular damage and cause mutations but are commonly used by immune cells to kill pathogens via an oxidative burst. Mounting evidence point to physiological signaling by ROS (Shadel and Horvath, 2015; Sies and Jones, 2020). H_2O_2 is favored as a physiological signaling molecule due to its higher stability, selective reactivity with cysteine groups and longer half-life. Similar to other second messengers, ROS can be induced by physiological stimuli, such as cytokines or mechanical forces (Droge, 2002). ROS operate in signaling through chemical reactions, which lead to covalent modifications through redox-sensitive cysteines (Brigelius-Flohe and Flohe, 2011; Ray et al., 2012; Kalyanaraman et al., 2018). Indeed, H_2O_2 is involved in engaging a number of signaling cascades (reviewed in Veal et al., 2007; Holmstrom and Finkel, 2014), such as tyrosine-protein kinases Lyn and Syk (Patterson et al., 2015), as well as affecting transcription factors (Marinho et al., 2014).

These redox-regulated protein switches can only operate in signaling if they are coupled to reducing enzymatic systems, such as thioredoxins (TDRXs) or peroxiredoxins (PDRXs) (Cox et al., 2009) [and possibly glutathione (GSH)]. Termination of ROS signals occurs through antioxidant enzymes – and probably

endogenous scavengers – which are likewise compartmentalized, with specific enzymes for mitochondria, cytosol, peroxisomes, and the extracellular space (Kaludercic et al., 2014).

Whilst large amounts of unregulated and non-compartmentalized ROS damage cells through oxidation of encountered proteins, lipids and nucleic acids, it has become increasingly apparent that ROS have physiological functions, such as intracellular signal transmission or as a feedback signal during monitoring of function. Key strategies to differentiate between sub-toxic and toxic ROS signals are to compartmentalize ROS, limit their amount, and control their diffusion capacity.

What to Do About/With So Many Radicals?

Mitochondrial ROS have two discernable fates: neutralization through antioxidants or release into the extramitochondrial space (**Figure 1**). The constant threat of excessive ROS generation and subsequent potential damage resulted in several antioxidant defense systems in mitochondria (Mailloux, 2018). SOD2 and GSH (Mari et al., 2009) are major ROS-inactivating mechanisms and supplemented with additional antioxidant machinery including GSH peroxidases (GPXs) (Handy et al., 2009), TRXPs, PRDXs, thioredoxin 2 (TRX2), glutaredoxin 2 (GRX2), and NAD(P)H/NAD(P)$^+$. Most antioxidants are located in the matrix, with the exception of PDRXs, which are located in the IMS to capture peroxides (such as H_2O_2) that are directed there or have escaped the matrix (Herrmann and Riemer, 2010; Sabharwal et al., 2013).

Reactive oxygen species from the IMS can affect both IMM and OMM proteins. Of particular consequence is the redox sensitive open probability of the abundant OMM VDAC that appears to be indirectly regulated by the OMM-anchored (2Fe-2S) mitoNEET or CDGSH iron sulfur domain 1 protein (Lipper et al., 2019). The phenomenon is central to superoxide-mediated apoptotic cytC release (Madesh and Hajnoczky, 2001).

SODs

Superoxide dismutases drive the reaction of two superoxide anions with two H^+ ions to generate the more stable H_2O_2 and water (Fukai and Ushio-Fukai, 2011; Hayyan et al., 2016). Though H_2O_2 can form •OH radicals, it now becomes available as a substrate for several antioxidant enzyme systems, including catalase, GPXs and PDRXs, so that it can be more efficiently removed. In humans, there are three SOD isoforms: SOD1 classically viewed as cytosolic and dependent on copper and zinc; SOD2, mitochondria-targeted and dependent on manganese (Karnati et al., 2013); SOD3, secreted and localized in the extracellular space and dependent on copper and zinc (Fukai and Ushio-Fukai, 2011; Hayyan et al., 2016).

An interrelationship between SOD2 and the ETC prevents unwarranted ROS generation and potential damage (**Figure 1A**). Loss of SOD2 results in decreased activity of CI and CIII, thus adapting to the availability of antioxidant. This phenomenon also highlights the importance of the SOD2 defense system, with losses neither compensated through other existing antioxidant systems nor via upregulation of cytosolic SOD1 (reviewed in Holley et al., 2011). Indeed, no change in SOD1 was observed in a kidney specific SOD2 knockout mouse model, wherein kidney function was unchanged (Parajuli et al., 2011).

ROS Neutralization by Imported Glutathione

Glutathione, the most abundant antioxidant, is synthesized through a two-enzyme reaction catalyzed by glutamate cysteine ligase and glutathione synthetase. It is a tripeptide nucleophile with thiol groups capable of accepting electrons in its reduced state (GSH), thereby becoming oxidized (GSSG), and rendering ROS to a lesser or non-reactive state (**Figure 1B**). Mitochondria cannot synthesize GSH *de novo*, yet harbor their own GSH pools (∼10 mM) (Kojer et al., 2012; Ribas et al., 2014), imported from the cytosol (Mckernan et al., 1991). The presence of mitochondrial glutathione reductase, together with NADPH, permits reduction of GSSG to GSH and its recycling. Intriguingly, the IMS GSH pool depends on cytosolic import through porins in the OMM whereas the matrix GSH pool does not (Kojer et al., 2012), implying tight regulation by reduction enzymes (e.g., glutathione reductase), with little exchange outside of the matrix.

REDOX CONTROL MECHANISMS THROUGH TRANSPORT AT MITOCHONDRIAL MEMBRANES

Though short-lived, ROS can exit the mitochondria to limit ROS accumulation or transmit signals to the extramitochondrial space. Spatially, ROS generated in the IMS are most likely to reach the cytosol where they function as signaling molecules or are degraded by catalases (predominantly in peroxisomes) to water and oxygen (**Figure 1A**). How can ROS pass through up to two lipid bilayer barriers whilst remaining intact and bypassing potential oxidative reactions? Since superoxide is charged and membrane diffusion of H_2O_2 is limited (Antunes and Cadenas, 2000; Cordeiro, 2014), membrane transporters are necessary and would offer protection from reactions. Anion channels could permit superoxide anions (Hawkins et al., 2007). In mitochondria, there are several anion channels (reviewed in Mackova et al., 2018). Though superoxide affects the activity of IMAC in the IMM and VDAC in the OMM, definitive evidence from electrophysiological studies for its conductance remains elusive.

Rather than direct superoxide conductance, superoxide could be dismutated to H_2O_2 by SOD2 and leave the matrix to the IMS by membrane diffusion or through AQP water channels (**Figure 1A**), so-called peroxiporins (Bienert et al., 2006; Bienert and Chaumont, 2014; Wang et al., 2020).

The major ROS species crossing the second membrane barrier between the IMS and cytosol is H_2O_2 (**Figure 1A**). The most obvious candidate for OMM H_2O_2 transfer is VDAC, since the OMM lacks AQPs, but no experimental evidence exists. A further consideration is the limited membrane diffusion of H_2O_2 that is rate limiting for release of ROS bursts to the cytosolic compartment, but possibly sufficient for signaling purposes. Why could H_2O_2 membrane diffusion occur at the OMM but not

at the IMM? Cytosolic and matrix H_2O_2 concentrations have been estimated at 80 pM (Lim et al., 2015) and 5–20 nM (Sies and Jones, 2020), respectively, and H_2O_2 may concentrate at cristae junctions, similarly to protons, to enhance the diffusion concentration gradient between IMS and cytosol. Other factors to take into consideration for influencing increased OMM H_2O_2 permeability are differences in molecular makeup of the two mitochondrial membranes. For instance, lower protein to lipid ratio of the OMM could increase the diffusion surface area, OMM lipid composition could affect lipid fluidity, OMM lipid fatty acid chain length decrease diffusion distance, or looser packing of OMM lipids permits thoroughfare of small molecules (Comte et al., 1976; Bienert et al., 2006).

TRANSPORT AT OMM AND IMM AND PROPERTIES IN RELATION TO METAL ION MOVEMENT

The OMM and IMM are functionally linked to maintain intramitochondrial spaces of defined ionic and proteinaceous composition. Achieving this requires a high degree of control by various transporters that are mainly expressed in the IMM. The IMM is structurally and functionally separated into tubular invaginations called cristae and the inner boundary membrane; these juxtapose the OMM and form the peripheral space.

It is assumed that OMM permeability is solely governed by the existence of relatively large pores, partially represented by VDACs or porins, which constitute approximately 50% of OMM proteins, allowing unregulated passage of small solutes intended for the mitochondrial matrix. VDACs permit a variety of negatively and positively charged ions as well as small organic molecules and metabolites up to 5 kDa to pass the OMM and traverse the IMS to reach the matrix (Colombini, 2016). VDAC can switch between different open or half-open/closed conformations and alternate between anion and cation conductive states (Tan and Colombini, 2007). Although it is not quite clear how VDAC switches between these states, some regulatory mechanisms have been recently described (reviewed in Lee and Thévenod, 2020).

In contrast, the IMM is highly selective due to the presence of an array of transport proteins that tightly regulate access to the mitochondrial matrix and in the opposite way to the IMS. Ion movement across the IMM requires uniporters, symporters and antiporters. They include the mitochondrial calcium uniporter (MCU) complex (Kamer and Mootha, 2015; Mammucari et al., 2017), the mitoferrin (Mfrn) 1/2 uniporters carrying Fe (II), Cu (II), and Mn (II), SLC25A37 and SLC25A28 (Paradkar et al., 2009), the Cu (II) transporter SLC25A3 (Boulet et al., 2018) and K^+ channels (Szabo et al., 2012; Augustynek et al., 2017). Furthermore, a K^+/H^+ exchanger (Zotova et al., 2010), various carriers for metabolites, including ANT (Palmieri and Monne, 2016), as well as translocases of the inner membrane (TIMs) that translocate proteins produced from nuclear DNA through these membranes for use by mitochondria (Pfanner et al., 2019) have been described.

The simplified view of OMM permeation involving VDAC has been challenged and complicated by recent reports identifying several OMM channel proteins and transporters (reviewed in Becker and Wagner, 2018), suggesting that the permeability of the OMM for electrolytes and small organic molecules is much more selective than previously thought. Moreover, we have identified the proton-coupled symporter DMT1 as a functioning OMM protein (Wolff et al., 2014a, 2018) (see section "A Recent, Versatile Candidate for OMM Import of Divalent Cations").

Is tight control of ion entry at the OMM level necessary, considering the selective permeability of the IMM to ions? At least for redox active essential metal ions, such as Fe (II), Mn (II), and Cu (I), it would make sense to coordinate their uptake at the OMM with their rate of entry at the IMM to avoid accumulation of these potentially harmful Fenton metal ions in the IMS. One could argue, however, that metal ion-induced oxidative stress in the IMS space is negligible because ROS are constantly produced as byproducts of the ETC (**Figure 1A**). Nevertheless, excessive ROS formation in the IMS, such as by accumulation of redox active metal ions, increases cytC release from mitochondria. Notably, a regulated transport pathway for redox active metal ions in the OMM, such as DMT1, would minimize that hazard. Moreover, an electrogenic H^+-driven transporter, such as DMT1 (Gunshin et al., 1997), at the OMM would represent an elegant mechanism to regulate uptake of Fe (II) for synthesis of Fe-sulfur clusters in the matrix that are required by the ETC complexes. Hence, the augmented proton-motive force generated by an increased ETC activity across the IMM would consequently slow down import of Fe (II) in a negative feedback loop.

A RECENT, VERSATILE CANDIDATE FOR OMM IMPORT OF DIVALENT CATIONS

To end in heme, Fe must also eventually cross the mitochondrial IMM, transfer that occurs, at least in part, via Mfrns (Paradkar et al., 2009), but what about the gap in movement from endosomes to there? Fe could enter the detectable, but difficult to define, labile Fe pool next (Cabantchik, 2014); or it might directly enter the mitochondria. Descriptions of how Fe entered into mitochondria after DMT1 driven endosomal exit in the last decade ranged from "not well understood" (Chen and Paw, 2012) with speculation about roles for two Fe chaperones designated PCBP1 and PCBP2 (Philpott and Ryu, 2014) to apparently unconstrained entry through the OMM from an Fe donor (Lill et al., 2012). They also included an intriguing docking of the organelles called "kiss and run" (Sheftel et al., 2007; Richardson et al., 2010; Hamdi et al., 2016), reviewed recently (Lane et al., 2015; Paul et al., 2017). Possibilities for the large pore that would be needed for entry include the (actually selective) VDAC or other porins (Colombini, 2016). The issue for iron can also be placed in the larger context of how selectively the OMM and IMM perform as barriers – debated above in section "Transport at OMM and IMM and Properties in Relation to Metal Ion Movement." We considered another mechanism when we detected mitochondrial DMT1 (Wolff et al., 2014b) closely associated with OMM markers and confirmed by subcellular fractionation. Additional support quickly followed (Wolff et al., 2014a), with both works reviewed subsequently

(Thévenod and Wolff, 2016). Later evidence showed that DMT1 was involved in import of Fe^{2+} and Mn^{2+} across the OMM (Wolff et al., 2018).

The initial paper (Wolff et al., 2014b) relied on a yeast-2-hybrid system to show that mitochondrial proteins, Tom6 and COXII, were potential DMT1 interaction partners. Co-immunoprecipitation of the latter with DMT1, and knowing that Parkin also did so (Roth et al., 2010), implicated DMT1 as a mitochondrial protein. DMT1 immuno-reactivity was detected in rat kidney cortex mitochondria by immunogold labeling (Wolff et al., 2014a). Mitochondria isolated from cultured epithelial cells and stably DMT1-transfected cells exhibited DMT1 immunoreactivity in Western blots that was substantially reduced or undetectable after stripping of the OMM; while DMT1 was enriched in OMM over mitochondria in images (Wolff et al., 2014b).

Experimental data in the latest paper (Wolff et al., 2018) indicated that increasing OMM DMT1 led to increased Fe uptake in isolated mitochondria by several criteria. Hence, DMT1 can alleviate rate-limited entry, ruling out unimpeded diffusion into mitochondria. Importantly, mitochondria isolated from kidneys of homozygous (*b*/*b*) Belgrade rats with a G185R mutation in DMT1 that reduces its transport capabilities had severely diminished uptake of Mn^{2+} in comparison to mitochondria from +/+ rats (with ones from +/*b*, intermediate), indicating that mitochondrial Mn^{2+} import is mostly dependent on DMT1. Two inhibitors of DMT1 blocked its activity in isolated mitochondria. While import of Mn^{2+} exhibited strong DMT1 dependence by several criteria, Fe^{2+} entry appeared to depend on not only DMT1 but also another route that currently remains unidentified.

Divalent metal transporter 1 is an H^+-cotransporter (Gunshin et al., 1997), a property that raises the role of pH gradients in mitochondria. Import of Mn^{2+} exhibited pH dependence (Wolff et al., 2018) like that found before (Garrick et al., 2006a). DMT1-dependent Fe^{2+}-induced quenching of an Fe indicator dye (Wolff et al., 2018) was stimulated by an inward-directed proton gradient (pH 6.2_o vs. pH 7.0_i) relative to an opposite pH gradient (pH 7.6_o vs. pH 7.0_i) with quenching unaffected for mitochondria equilibrated at pH 6.2 or 7.6, excluding an unspecific pH effect. Interestingly, no such pH gradient dependence has been observed for the IMM Fe carrier Mfrn1 from the cichlid *Oreochromis niloticus* reconstituted in proteoliposomes (Christenson et al., 2018). Moreover, Mfrn1-mediated Fe uptake was substantially diminished below an external pH of 6.5. Although this value is lower than what has been determined for the IMS (pH 6.88) in mitochondria from an endothelial cell line (Porcelli et al., 2005), IMS pH in cells *in vivo* may well be lower due to stronger dependence on mitochondrial energy metabolism (cf. below). Thus, DMT1 and Mfrns may conceivably cooperate in Fe^{2+} delivery to the mitochondrial matrix relying on acidic IMS pH.

Although some might consider the descriptions for OMM entry to be mechanistic alternatives, we note that one (Chen and Paw, 2012) was really a statement acknowledging the field's status; while another (Lill et al., 2012) can be treated as implying that absent a candidate for a gateway is equivalent to the absence of a gateway. We regard it as nearly self-evident that an organelle that manages O_2, unavoidably generates ROS and needs transition metals to carry out its functions necessarily regulates entry of their ions. The third description (Richardson et al., 2010) clearly includes regulation. Transient docking of endosomes and mitochondria that allows Fe to move from the Tf-Tf receptor complex to within the intermembrane space of the mitochondria without a presence in the cytosol, can also involve DMT1 on the OMM. One could jokingly in part refer to DMT1, also present on the endosomal membrane, as potentially the lips of the kiss by considering the possibility that DMT1 on the surface of the two organelles associates to help docking occur. This mechanism would provide a tunnel so that Fe^{2+} does not exist in a state where it is freely exposed to the cytosol in transit. It is important to note that participation of DMT1 in import of metal ions into mitochondria and argument for docking of the two organelles rely on independent observations and either mechanism could be validated or challenged without the other hypothesis necessarily rising or falling as well. For example, "kiss and run" receives strong support in erythroid precursors where heme biosynthesis demands high Fe flux (Hamdi et al., 2016) but the strongest non-erythroid evidence supporting the hypothesis comes from experiments on MDCK-PTR cells (Das et al., 2016). Blocking intra-endosomal Fe release increased both duration of the two organelles' interactions and motility of Tf-endosomes, which may provide inspiration for experiments on whether OMM DMT1 participates in the same process. Although this cell line was derived from a canine kidney, its use is primarily noteworthy for showing that erythroid commitment to extraordinary Fe flux into heme need not be a requirement for organelle docking to prevent ROS. One virtue of coordinating the two hypotheses to make DMT1 the lips of the kiss is that the endosome will expel protons to drive Fe^{2+} or Mn^{2+} across its membrane into the OMM and perhaps through Mfrn into the mitochondrial matrix.

HOW DOES DMT1 INTEGRATE INTO MITOCHONDRIAL METAL HOMEOSTASIS?

The data referenced (Wolff et al., 2014a, 2018) support a role for DMT1 in import of Fe^{2+} and Mn^{2+} across the OMM; however, much more needs to be learned to integrate it fully into how the organelle handles metal ions. While DMT1 probably accounts for most Mn influx, an unidentified participant adds to Fe influx. Because DMT1 can handle multiple other metals (Gunshin et al., 1997; Garrick et al., 2006b; Illing et al., 2012), identifying for which ones its role is significant at the OMM remains to be determined. Hence, integration for many metals can only be speculative. Nevertheless, we must add Cd, a toxic metal with a high DMT1 affinity as relevant for this review. Presumably, Cd toxicity derives from its not having Fenton chemistry, with it usually considered not to be a transition metal.

A substantial portion of metal metabolism occurs within the IMM after DMT1 transport across the OMM. Mfrn 1/2 clearly take over for Fe^{2+} (Chen and Paw, 2012) at the IMM with the former involved in Fe crossing erythroid IMMs while its paralog does this for non-erythroid IMMs. Mfrn 1 is capable of transporting Mn and some other DMT1 substrates

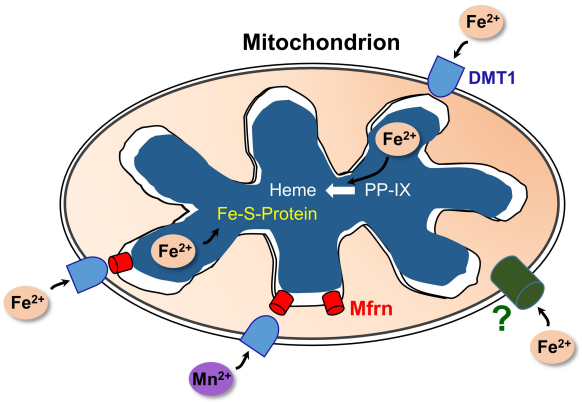


FIGURE 2 | A model of DMT1 participating in mitochondrial iron homeostasis. Keys: DMT1, mitoferrins (Mfrn), ferrous iron (Fe^{2+}), Mn^{2+}, iron-sulfur cluster proteins (Fe-S-Protein), protoporphyrin IX (PPIX), and heme. Fe^{2+} enters the OMM via DMT1 and an as-yet unidentified pathway (?); while Mn^{2+} apparently relies primarily on DMT1 for import. After entry, the two cations could enter the IMS then pass through Mfrn (lower right) or move directly through Mfrn (lower left). Adapted and redrawn with permission from Garrick and Thévenod (2019).

(Christenson et al., 2018) but whether it or its counterpart play that role at the IMM is not yet known. Another question that having DMT1 control movement of divalent metals across the OMM raises relates to the metals' role in generating and managing free radicals. DMT1 binds the Fe chaperone PCBP2 (Yanatori et al., 2014), transferring Fe directly to it. Is the IMS insubstantial in metal homeostasis? Or is there a need for chaperones like PCBP1 or 2? For Cu the presence of SOD1 and multiple other Cu-related proteins there (Baker et al., 2017) sets a precedent that may well apply to other members of the metallome.

Selected aspects of how DMT1 could participate in mitochondrial metal metabolism are represented in **Figure 2**. Other aspects, omitted to avoid making the representation too complicated, include the influence of proton gradients, possible coordination with endosomal docking to mitochondria that might create a tunnel from endosomes to mitochondrial matrix, possible chaperones in the IMS and potential distinctions between occurrences in renal mitochondria and other specialized mitochondria like erythroid ones.

SYSTEMIC AND CELLULAR IRON HOMEOSTASIS

Newly recognized mitochondrial (including renal mitochondria) acquisition of iron via OMM DMT1 needs to be set the broader context of systemic and cellular iron homeostasis (Hentze et al., 2010; Garrick, 2016; Wang et al., 2019; Yanatori and Kishi, 2019). Systemic iron metabolism in humans begins with uptake of iron from the diet in the duodenal lumen where it presents as Fe^{3+} (**Figure 3**, left). Hence, a ferrireductase must generate Fe^{2+} with DCYTB, representing Duodenal Cytochrome B, also known as cytochrome b reductase 1, encoded by CYBRD1. DMT1 is the major Fe^{2+} importer, acting as a proton symporter. The inward proton gradient is maintained by NHE3, a Na^+/H^+ antiporter. Arriving in the enterocyte, a polarized epithelial cell, Fe^{2+} follows two main pathways: either vectorial for systemic distribution, i.e., transcellularly to exit (the main iron-related function as the source of nearly all systemic Fe), or cellular by entering the mitochondrion or nucleus, storage in ferritin, or other cell usages. Chaperones like PCBP's aid in minimizing ROS. Fe^{2+} exit depends on oxidation to Fe^{3+} by Hephaestin (HEPH) or possibly other ferroxidases coordinated with export by Ferroportin (FPN1); Fe^{3+} then interacts with apo-Tf to load the Tf to mono- or di-ferric Tf in the circulation, representing Tf bound Fe (TBI).

Cellular iron homeostasis is well embodied by erythroid precursors because a substantial majority of iron acquisition leads to hemoglobin synthesis (**Figure 3**, right). They incorporate Fe delivered by Tf from the circulation. Fe-Tf or Fe_2-Tf bind to the ubiquitous Tf-receptor 1 (TfR1) that is present on the surface of immature erythroid and other cells where the complex undergoes endocytosis. The endosome formed by this process acidifies due to the activity of a vacuolar H^+-ATPase, supplying protons to allow the Tf-TfR1 complex to release Fe^{2+} in cooperation with endosomal reductase. Relieved of its Fe, apo-Tf-TfR1 exocytosis to the cell surface; at the higher pH there, the complex releases apo-Tf to the circulation to retrieve more Fe. The H^+ also drive DMT1 as an endosomal exit transporter to supply the iron to mitochondria as discussed in sections "A Recent, Versatile Candidate for OMM Import of Divalent Cations" and "How Does DMT1 Integrate Into Mitochondrial Metal Homeostasis?" The vast majority of Fe flux in erythroid mitochondria goes into heme synthesis needed for hemoglobin formation, but non-erythroid cells have a more even balance between heme synthesis and non-heme iron utilization such as in iron-sulfur proteins. For its cellular regulation, iron depends largely on an iron response element-iron regulatory protein system (IRE-IRP) (reviewed in Hentze et al., 2010; Papanikolaou and Pantopoulos, 2017).

When mature erythrocytes become effete, nearly all their iron is recovered by macrophage (or hepatic Kupffer cells) that may store it temporarily, but ultimately resupply most of it to erythroid precursors. The liver also serves as a temporary depot via the portal circulation and TBI constitutes the usual physiological source of iron for most cells where metabolism resembles the erythroid cell except that demand for heme biosynthesis is modest. With no regulated means for iron depletion, excellent renal recovery (see below) and competent recycling, the onus is on the duodenum (and recovery systems) to prevent systemic iron overload and to withhold iron during inflammation. How hepcidin, a master regulatory peptide, accomplishes these goals is well covered in reviews (Hentze et al., 2010; Wang et al., 2019; Camaschella et al., 2020).

Much of the current review concerns renal management of systemic iron metabolism by recovering nearly all filtered forms of iron. Indeed, the kidney has emerged as an important physiological organ that contributes to regulation of systemic Fe levels (Smith and Thévenod, 2009). In addition to usage of reabsorbed Fe in cellular homeostasis, polarized epithelial kidney

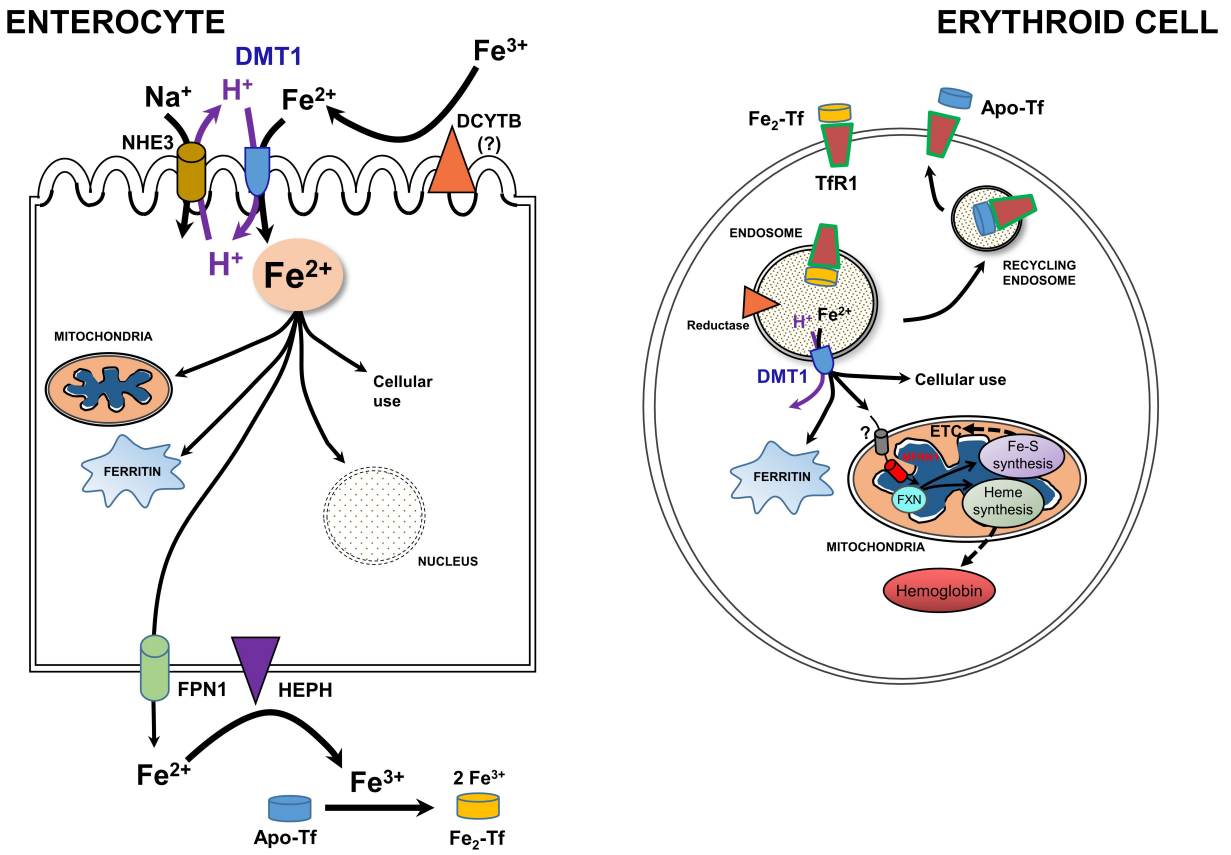


FIGURE 3 | A model for integration of cellular iron homeostasis with systemic iron homeostasis. Keys: Enterocytes, iron (Fe^{3+} & Fe^{2+}), a ferrireductase (DCYTB); "?" indicates that it is unlikely to be the only reductase), DMT1, NHE3, a mitochondrion, ferritin, the nucleus, Hephaestin (HEPH), Ferroportin (FPN1) and Na^{+}/K^{+} antiporter. Not shown are tight junctions with other enterocytes and the Tf-receptor that likely binds apo-Tf so that it can be loaded with Fe. Also keyed: Erythroid cells, TfR1. di-ferric Tf binding to TfR1, apo-Tf released by it, endocytosis -making an endosome to go through the Tf-cycle (see text), an endosomal reductase, an ATPase, DMT1, cytosolic ferritin, mitochondrial components, and metabolism including Mfrn1 = erythroid mitoferrin, FXN – frataxin, ETC and Fe-S plus Heme synthesis and hemoglobin formation. Adapted and redrawn from Wang et al. (2019) with permission from Oxford University Press on behalf of the American Society for Nutrition.

cells prevent Fe loss from the body by vectorial transport of loosely bound Fe and by receptor-mediated endocytosis of high- and low-affinity Fe-binding proteins (such as Tf or albumin, respectively) into the blood (Langelueddecke et al., 2012; Smith et al., 2019) (reviewed in Thévenod and Wolff, 2016). Fe transport mechanisms have also been identified in the kidney that depend on systemic and/or locally produced hepcidin levels as well as on IRE-IRP system dependent and independent transporters (reviewed in Thévenod and Wolff, 2016; Van Swelm et al., 2020; see **Figure 4A**).

Fe OVERLOAD AND RENAL INJURY

Disturbances in cellular and systemic Fe balance contribute to kidney injury either by initiating oxidative stress and mitochondrial dysfunction or by modulating inflammatory processes (Thévenod and Wolff, 2016; Van Swelm et al., 2020). Fe overload of the kidney occurs by increased filtration of Fe, in the form of TBI and NTBI, either due to systemic Fe overload or kidney disorders. Hemochromatoses and dyserythropoiesis underlie mostly systemic Fe overload of genetic origin whereas acquired systemic Fe overload usually occurs through transfusions (Brissot et al., 2019). Renal diseases causing Fe overload of the kidney involve proteinuria of glomerular origin, that predominantly causes PT damage, or decreased PT reabsorption (e.g., as in Fanconi syndrome due to PT damage) with Fe overload of downstream nephron segments. Overload occurs because the PT (in particular the S1-segment) (Schuh et al., 2018) is equipped with both a receptor for (metallo-)protein endocytosis, megalin:cubilin:amnionless (Kozyraki et al., 2001; Weyer et al., 2011) and endo-lysosomal machinery expressing DMT1 (Abouhamed et al., 2006), promoting Fe accumulation and damage of PT cells. Distal nephron segments take up Fe via DMT1 in their apical membranes (DCT > thick ascending limb of LOH > CD) (Ferguson et al., 2001). The protein receptor SLC22A17 [with higher affinity to protein ligands, such as Tf, neutrophil gelatinase-associated lipocalin (NGAL) / lipocalin-2 and albumin, than megalin:cubilin:amnionless] engulfs Fe-protein complexes in DCT and CD (Langelueddecke et al., 2012).

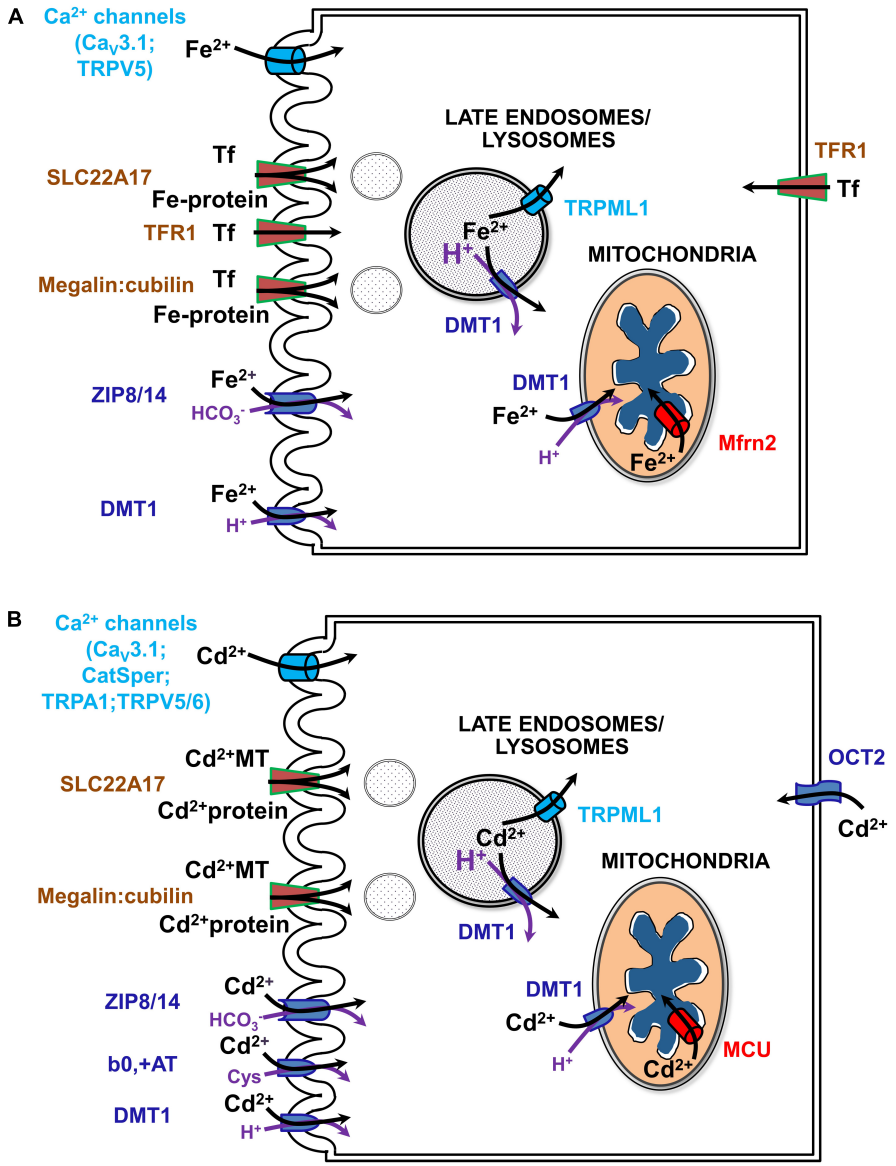


FIGURE 4 | Uptake pathways for Fe **(A)** and Cd **(B)** via channels, solute carriers (SLCs) and receptors in renal epithelial cells. See text and (Thévenod and Wolff, 2016; Thévenod et al., 2019; Van Swelm et al., 2020) for further details. b0,+AT, amino acid/cystine transporter (SLC7A9/SLC3A1); CatSper, cation channel of sperm; Cav3.1, voltage-gated calcium channel alpha subunit 3.1; DMT1, divalent metal transporter 1 (SLC11A2); MCU, mitochondrial calcium uniporter; megalin:cubilin, the megalin:cubilin:amnionless complex; Mfrn2, mitoferrin 2 (SLC25A28); OCT2, organic cation transporter 2 (SLC22A2); SLC22A17, NGAL/lipocalin 2 receptor; Fe-Tf, holo-transferrin; TfR1, Tf receptor 1; TRPA1, transient receptor potential cation channel subfamily A member 1; TRPML1, transient receptor potential cation channel subfamily ML member 1; TRPV5/6, transient receptor potential cation channel subfamily V members 5/6; ZIP8/14, zinc transporters 8/14 precursors (SLC39A8/A14).

Increased uptake of Fe via these transporters and receptors may induce nephrotoxicity (Thévenod and Wolff, 2016; Van Swelm et al., 2020). Fe overload has been described in the distal nephron of hemochromatosis mouse models (Moulouel et al., 2013). Hemolysis or rhabdomyolysis can also lead to increased glomerular filtration of heme, hemoglobin or myoglobin, which are taken up by both PT and the distal nephron (Moulouel et al., 2013) via megalin:cubilin:amnionless (Gburek et al., 2002) and SLC22A17 (Van Swelm et al., 2018). Interestingly, urinary tract infection decreases Fe accumulation in the distal nephron of hemochromatosis mouse models (Houamel et al., 2016) along with decreased SLC22A17 expression (Betten et al., 2018), supporting a role for SLC22A17 in mediating Fe uptake in the distal nephron. In addition to apical exposure, tubular cells should also be exposed to increased circulating Fe levels at their basolateral side during Fe overload conditions.

Importantly, alterations of cellular Fe homeostasis pathways involving accumulation of damaging redox active labile Fe in

relevant nephron segments may elicit Fe-mediated renal cell injury or death and play a causative role in the pathogenesis of AKI (and possibly CKD) (reviewed in Scindia et al., 2015; Swaminathan, 2018; Van Swelm et al., 2020). Necroptosis (Van Swelm et al., 2018) and possibly two other forms of regulated cell death, ferroptosis and pyroptosis, have been implicated in AKI induced by Fe (reviewed in Van Swelm et al., 2020), but evidence is weak here, and no data are available for the mode(s) of cell death involved in CKD. Fe-dependent AKI may result from increased renal exposure to heme, myoglobin and/or from ischemia–reperfusion injury because release of catalytic Fe from damaged renal tubular epithelial cells into the tubule lumen may result in exposure of neighboring cells. Fe either initiates oxidative stress with ROS formation and mitochondrial dysfunction, mainly by inducing the mPTP (see sections "Apoptosis Signaling" and "Cd and Kidney Mitochondrial Damage") or is a potent modulator of inflammation. Persistence of these harmful pathological processes is thought to drive AKI-to-CKD progression (Liu et al., 2018), which is supported by the observation of increased accumulation of Fe in tubular epithelial cells and infiltrating macrophages in animal models of CKD and in kidney biopsy samples from patients with CKD (reviewed in Van Swelm et al., 2020). Moreover, clinical studies indicate an association between Fe overload and CKD (Slotki and Cabantchik, 2015) that may involve mitochondrial damage (see section "Fe and Mitochondrial Damage").

Fe AND MITOCHONDRIAL DAMAGE

Kidney tubular epithelial cells are vulnerable to oxidative stress due to their energy demand and high numbers of mitochondria (Liu et al., 2018). Yet data on damaging effects of Fe on renal mitochondria are scarce (**Figure 1B**). Several models of AKI by acute Fe overload of the kidney show consistently damaged kidney mitochondria induced by increased mitochondrial Fe and oxidative stress. Twenty hours after a single intraperitoneal injection of iron-dextran (500 mg/kg body weight) into rats, increased mitochondrial Fe correlated with increased lipid peroxidation and decreased mitochondrial α-tocopherol content in cortex and medulla (Galleano et al., 1994), whereas other mitochondrial functions were unaffected. Thirty minutes to 3 h after intraperitoneal injection of Fe- nitrilotriacetic acid (10 or 20 mg Fe/kg body weight), mitochondria of rat kidney PT cells showed increased oxidative damage, supported by accumulation of 4-hydroxy-2-nonenal-modified proteins, indicating oxidative breakdown of polyunsaturated fatty acids and related esters (Zainal et al., 1999). In rat kidneys, myoglobin Fe released into the circulation by experimental rhabdomyolysis produces oxidative stress and mitochondrial dysfunction through lipid peroxidation of the mitochondrial membranes (Plotnikov et al., 2009). Inhibitors of mPTP, mitochondria-targeted antioxidant (SkQ1), and Fe chelation with deferoxamine abrogated release of cytC as well as defective ETC and OXPHOS. In a subsequent study of myoglobinuric AKI in rats, cell death occurred with sustained caspase 3 cleavage (Funk and Schnellmann, 2012). Expression of mitochondrial fission protein Drp1 and the fusion protein Mfrn2 were elevated as well as markers of mitochondrial biogenesis (PGC-1α and PGC-1-related co-activator). Respiratory proteins NADH:Ubiquinone Oxidoreductase Subunit B8, ATP synthase β, cytC oxidase subunit I (COX I), and COX IV were decreased, indicating persistent disruption of mitochondrial homeostasis even in the presence of mitochondrial recovery signals (Funk and Schnellmann, 2012). Postoperative AKI caused by hemoglobinemia, has also been associated with an enhanced oxidative stress response and lipid peroxidation (Billings et al., 2011).

Fe-induced excessive formation of mtROS and lipid peroxidation inhibit ETC activity (Musatov and Robinson, 2012). In addition, increased ROS formation depletes mitochondrial antioxidants, such as GSH, or damage anti-oxidative defense enzymes, as described in liver mitochondria (Jagetia and Reddy, 2011; **Figure 1B**). Clinical studies indicate an association between Fe overload and CKD (Slotki and Cabantchik, 2015). Mitochondrial damage induced by increased Fe has been implicated in the pathophysiology of CKD. Damage involves a decrease in frataxin, a mitochondrial protein possibly involved in assembly of Fe-sulfur clusters and acting as an Fe-binding protein (Pastore and Puccio, 2013). Moreover, accumulation in mitochondria of 5-aminolevulinic acid, a heme precursor that increases oxidative stress and has direct disruptive effects on mitochondrial function has been discussed (reviewed in Nakanishi et al., 2019). Nevertheless, the evidence linking these molecular pathologies with CKD is correlative.

Interestingly, loss of SOD2 activity culminates in Fe accumulation in mitochondria accompanied by increases in regulators of the Fe pool: ferritin, transferrin receptors, transferrin, hepcidin, and frataxin (reviewed in Holley et al., 2011). Furthermore, SOD2 knockdown in erythroid cells results in reduction of the IMM transporter ABCB7 (Martin et al., 2011), which exports Fe-sulfur centers to the cytosol for synthesis of Fe-sulfur center containing proteins. Moreover, ABCB7 downregulation results in mitochondrial Fe overload as well as diminished SOD2 activity (Cavadini et al., 2007). The main alterations of mitochondrial structure, function and expression elicited by increased mitochondrial Fe are summarized in **Table 1** (left).

Cd TOXICITY AND THE KIDNEY

Due to enhanced industrial and agricultural activities, Cd accumulates in the environment. Hence, Cd is one of the top 20 hazardous substances worldwide and a significant public health issue (Faroon et al., 2012). Further, chronic low Cd exposure (CLCE) is a health hazard for ∼10% of the world population with increased morbidity and mortality (Jarup and Akesson, 2009; Moulis and Thévenod, 2010).

Food and cigarette smoking are the prime sources of CLCE (Satarug and Moore, 2004). For non-smokers, food grown in Cd-containing rock phosphate fertilizers is the major source of CLCE (Pan et al., 2010). Bioaccumulation of Cd in plants, including tobacco, is the first step in the human food chain that results

TABLE 1 | Key alterations of mitochondrial structure, function and expression elicited by increased mitochondrial Fe and Cd transport.

	Fe		Cd	
Oxidative damage (lipids; proteins)	+	Galleano et al., 1994; Zainal et al., 1999; Plotnikov et al., 2009; Billings et al., 2011; Musatov and Robinson, 2012	+	Reviewed in Lee and Thévenod, 2020
Depletion of antioxidants (GSH; α-tocopherol)	+	Galleano et al., 1994	+	Reviewed in Lee and Thévenod, 2020
Damage of antioxidative enzyme defense (↓ function and/or expression)	n.d.		+	Reviewed in Cuypers et al., 2010
Disruption of ETC, OXPHOS and $\Delta\psi_m$	+	Plotnikov et al., 2009; Musatov and Robinson, 2012	+	Belyaeva et al., 2004; Wang et al., 2004; Lee et al., 2005a,b
Mitochondrial permeability transition pore (mPTP)	+	Plotnikov et al., 2009	+	Belyaeva et al., 2002, 2004
Activation of apoptotic signaling	+	Plotnikov et al., 2009; Funk and Schnellmann, 2012	+	Lee et al., 2005a,b; Nair et al., 2015
Changes in fusion-fission dynamics and biogenesis	+	Funk and Schnellmann, 2012	+	Nair et al., 2015
Alterations of mitochondrial DNA levels	n.d.		+	Nair et al., 2015
Decreased expression of respiratory proteins	+	Funk and Schnellmann, 2012	+	Reviewed in Lee and Thévenod, 2020
Decreased heme and Fe-sulfur cluster synthesis	+	Pastore and Puccio, 2013; reviewed in Nakanishi et al., 2019	n.d.	
Structural and membrane defects	+	Zainal et al., 1999	+	Kerek and Prenner, 2016; Reviewed in Lee and Thévenod, 2020

Observed effects are also dependent on time of exposure and metal ion concentrations used. For additional details and references, see sections "Fe and Mitochondrial Damage" and "Cd and Kidney Mitochondrial Damage." n.d., not determined.

in Cd accumulation in the body. Various organs and systems are affected by CLCE, causing kidney damage, osteoporosis, genotoxicity, teratogenicity, or endocrine, and reproductive defects (Jarup and Akesson, 2009). Cd in tobacco smoke is an independent risk factor in the development of smoking-associated chronic diseases, such as cardiovascular disorders and cancer (Abu-Hayyeh et al., 2001; Huff et al., 2007).

The kidneys contain ∼60% of the Cd body burden in the age range of 30–60 years (Salmela et al., 1983; Jarup and Akesson, 2009). Cd accumulates in the kidneys, in particular the PT, because intracellular Cd induces the upregulation of the protein MT, rich in sulfhydryl (SH) groups that bind the metal ion with high affinity and thereby prevent its toxic effects (Freisinger and Vasak, 2013). Although Cd buffering by MT is initially protective for the kidneys, it in fact represents a dangling sword of Damocles because Cd accumulation over time develops into an endogenous source of high concentrations of potentially toxic Cd and is associated with chronic kidney disease (Gibb et al., 2019; Satarug et al., 2020).

The question remains how Cd enters mammalian cells in general, and particularly kidney cells, because no physiological process requires the non-essential toxic-only ion Cd. Simply, Cd imitates essential metal ions such as Fe, Mn, Zn, Ca, and Cu, and crosses cell membrane barriers by competing for their transport pathways (Thévenod et al., 2019; see also **Figure 4B**). To describe this process, the term "ionic and molecular mimicry" has been created (Clarkson, 1993; Bridges and Zalups, 2005). Nevertheless and unfortunately, Cd ionic mimicry does not hold for the only known cellular Fe efflux transporter FPN1 (Mitchell et al., 2014) which is expressed basolaterally in kidney PT cells (Wolff et al., 2011) and does not allow Cd to exit renal cells. The inability of FPN1 to transport Cd for vectorial reabsorption into the circulation (in contrast to Fe, Co, and Zn) may partly explain major Cd accumulation and toxicity of renal cortical PT cells (see above).

By binding to essential side groups of biomolecules (e.g., SH groups) and/or displacing essential metals from macromolecules, Cd disrupts cellular functions with subsequent death or disease (Moulis, 2010; Thévenod and Lee, 2013). Cd interacts with proteins by substituting for zinc ions, as in enzymes or transcription factors (Maret and Moulis, 2013; Petering, 2017), or replacing calcium in cellular signal transduction, interfering with thiol-dependent redox systems, and modifying second messenger levels, growth and transcription factors (Thévenod, 2009; Thévenod and Lee, 2013). Cd is not a transition-metal ion undergoing Fenton chemistry, yet in biological systems, it indirectly increases ROS and RNS. Cd depletes endogenous anti-oxidative ROS scavengers (such as GSH), affects ROS-producing/metabolizing anti-oxidative enzymes, disrupts mitochondrial function, in particular the mitochondrial ETC (see also **Figure 1C**), and/or displaces redox active metals, such as Fe or copper (Cuypers et al., 2010). Cd damages DNA indirectly by causing increased ROS formation and therefore has been classified as a class I carcinogen; it also interferes with major DNA repair systems and inactivates tumor suppressor functions by targeting zinc-finger proteins. This effect may cause genomic instability and promote tumor initiation and progression (Hartwig, 2013).

Cd AND KIDNEY MITOCHONDRIAL DAMAGE

Mitochondria not only traffic and redistribute within the cell in response to local energy demands, but are also dynamic organelles that continuously fuse and divide to maintain functional mitochondria when cells experience metabolic or environmental changes (Youle and Van Der Bliek, 2012). Moreover, chronic stress and high metabolic demand induce mitochondrial biogenesis, which is partially regulated by the

transcription factors peroxisome proliferator-activated receptor (Ppar) and its coactivator 1 (Pgc-1) (Scarpulla, 2012; Dorn, 2019).

Cd damages mitochondrial structures and membranes in kidney cortex by reducing cristae number, inducing cristae shortening and reduced expression of COXs (reviewed in Lee and Thévenod, 2020). Cd interacts with cardiolipin, a mitochondrial phospholipid localized in the IMM, in artificial liposomes, increasing membrane rigidity (Kerek and Prenner, 2016), and thereby possibly enhances cytC release for apoptotic signaling.

Cd affects renal mitochondrial biogenesis concentration dependently, both *in vitro* and *in vivo* (Nair et al., 2015). At 1-10 μM $CdCl_2$ for 24 h, Pparγ and mitochondrial DNA (mtDNA) were augmented in cultured renal PCT cells whereas Pparα and Pgc-1β were not affected, occurrences correlated with minor or low loss of GSH and low rates of apoptosis. In contrast, 30 μM $CdCl_2$ attenuated Pparα, Pgc-1β and mtDNA, despite sustained Pparγ increase, and was associated with increased oxidized GSH GSSG and pro-apoptotic markers. Similarly in sub-chronically $CdCl_2$-treated rats (1 mg/kg/day, s. c., 2 weeks), Pparα and mtDNA significantly increased whereas GSH was unchanged compared to saline-treated controls (Nair et al., 2015), indicating that mitochondrial biogenesis is part of an adaptive mechanism to chronic oxidative stress by Cd.

How does Cd permeate mitochondria? Cd may mimic calcium at binding sites due to their similar hydrated ionic radii (Marcus, 1988). Hence Cd could permeate the OMM via VDAC in its half-open/closed state, similarly as described for calcium (Colombini, 2016). Alternatively, Cd may cross the OMM through DMT1, given that it is a known substrate (Thévenod et al., 2019) (see sections "A Recent, Versatile Candidate for OMM Import of Divalent Cations" and "How Does DMT1 Integrate Into Mitochondrial Metal Homeostasis?"). Because Cd permeates certain types of Ca^{2+} channels, e.g., voltage gated T-type calcium channels or TRPV5/6 (reviewed in Choong et al., 2014), Cd permeation of the IMM may occur through the MCU complex, the major IMM Ca^{2+} channel (Kirichok et al., 2004; Thévenod et al., 2019). Indeed, as shown in isolated mitochondria from kidney cortex (as well as from liver) pharmacological MCU inhibitors ruthenium red, Ru360 or La^{3+} abolished Cd-induced mitochondrial dysfunction, such as swelling, loss of membrane potential and pro-apoptotic cytC release, consistent with Cd transport through the MCU into the matrix (Dorta et al., 2003; Li et al., 2003; Lee et al., 2005a,b).

To adjust to cellular energetic demands, mitochondria swell, and contract through monovalent cation cycling to regulate matrix biochemical reactions, such as β-oxidation, Krebs cycle activity and respiration that are decreased by mitochondrial swelling (Lizana et al., 2008; Nowikovsky et al., 2009). Isolated energized rat kidney cortex mitochondria suspended in KCl buffer undergo swelling followed by rapid contraction after addition of 5–20 μM $CdCl_2$, whereas non-energized mitochondria do not contract (Lee et al., 2005b). Using pharmacological inhibitors, we showed that Cd entered via the MCU and elicited $\Delta\psi_m$ driven K^+ influx via a K^+ uniporter to induce matrix swelling, with subsequent activation of a quinine-sensitive K^+/H^+-exchanger, resulting in mitochondrial contraction (prior to dissipation of $\Delta\psi_m$) (Lee et al., 2005b). Hence, transient mitochondrial swelling by Cd may be part of an adaptive stress response, together with a temporary switch in energy metabolism, that precedes mitochondrial dynamics and removal of damaged mitochondria by mitophagy.

Once in mitochondria, Cd interferes with the function of the ETC to dissipate Δψm (Belyaeva et al., 2004; Lee et al., 2005b; **Figure 1C**). Cd (20 μM) inhibited ETC complex activities from various organs (CIII > CII >CI > CIV), maximally by ∼75% (Wang et al., 2004). CIII catalyzes the transfer of electrons from ubiquinol to cytC, and Cd competes at the zinc-binding site, preventing electron transfer and resulting in increased superoxide.

Intriguingly, CI can produce ROS through reverse electron transfer (RET) during low ATP demand and a large proton-motive force can drive electrons to flow to CI, leading to superoxide formation. RET depends on the proton-motive force and oxidative status of mitochondrial coenzyme Q and NADH pools (Robb et al., 2018). Could RET be relevant to Cd-induced oxidative stress? ETC inhibition by Cd will hinder electron shuttling, increasing NADH oxidation (Cameron et al., 1986) as well as dissipating $\Delta\psi_m$. These would result in either loss of the reduced NADH pool and/or matrix-directed proton-motive force, and quite plausibly drive RET and superoxide production through CI. These effects could enhance the loosely bound cytC pool and result in increased cytC apoptogenicity (Petrosillo et al., 2003). Loss of ATP generation by Cd through the ETC could be an important additional step in the transformation progression of normal cells, ultimately making the switch to glycolysis, also known as the Warburg effect, which is a hallmark of cancer cells (Gogvadze et al., 2009).

Similar to other apoptotic stimuli Cd dissipates $\Delta\psi_m$, possibly involving the mPTP, resulting in mitochondrial dysfunction and apoptosis (see section "Apoptosis Signaling"). Uncertainty about the molecular signature of mPTP is paralleled by conflicting studies addressing the question of whether Cd induces mPTP opening as part of its cell death signaling (Belyaeva et al., 2002; Li et al., 2003; Lee et al., 2005a). No evidence for Cd induction of mPTP was found in studies on Cd-induced swelling of isolated mitochondria from rodent kidney monitored by light scattering measurements in combination with known pharmacological modulators of the originally postulated mPTP components (cyclosporin A, bongkrekic acid, atractyloside) (Li et al., 2003; Lee et al., 2005a). Ineffectiveness of pharmacological modulators, such as cyclosporin A, in preventing Cd-induced swelling of isolated kidney and liver mitochondria, indicates that the mPTP is not "the" ubiquitous mitochondrial swelling mechanism elicited by Cd (reviewed in Lee and Thévenod, 2006). Indeed, opening of an IMM water channel, AQP8, by Cd is a likely entryway of water influx into the matrix to cause swelling of kidney (and liver) mitochondria (Calamita et al., 2005; Lee et al., 2005a; Lee and Thévenod, 2006). Interestingly, mammalian AQP8 appears to have the largest H_2O_2 permeability (Bienert et al., 2007). Genetic manipulation of mitochondrial AQP8 supports its role in H_2O_2 release to the cytosol (Danielli et al., 2019a,b), which could contribute to Cd-induced cell death.

Cd could activate mitochondrial AQP8 directly by binding at calcium binding sites found on AQPs (Fotiadis et al., 2002),

or indirectly by modulating the biophysical properties of the IMM lipid bilayer, as demonstrated for other AQPs and cellular organelles (Tong et al., 2012). Hence, it is attractive to speculate that an increase in mitochondrial membrane fluidity by Cd could enhance AQP8 activation.

An additional mechanism for Cd-induced mitochondrial damage due to increased ROS formation may be interference with mitochondrial antioxidative defense enzymes (**Figure 1C**), leading to decreased expression or reduced functional activity (e.g., by displacement of redox active metal ions from these enzymes, or by complex formation of Cd with sulfhydryl groups). It could involve mitochondrial MnSOD, SOD2, Fe (III)-containing catalase, selenocysteine-containing GPX1 (reviewed in Cuypers et al., 2010), and possibly mitochondrial GSH and glutathione reductase (Ribas et al., 2014).

Cd and Fe damage are compared in **Table 1**. Strikingly, despite the Fe undergoing Fenton chemistry and Cd not, there is a significant overlap of alterations of mitochondrial structure, function, and expression elicited by increased mitochondrial Fe and Cd, possibly because of displacement of Fe by Cd (see above).

THERAPY

The best and paramount therapy of Fe or Cd nephrotoxicity is its prevention (reviewed in Nawrot et al., 2010; Wish et al., 2018). Apart from symptomatic treatment, there is currently no effective clinical therapy for acute Fe or Cd intoxication. Two treatment approaches found their way in the literature: (1) metal chelating agents without or (2) with antioxidants. The Fe chelating agents currently in use for prevention and management of AKI include deferoxamine, deferasirox and deferiprone, all of which are highly specific for Fe (Sharma and Leaf, 2019). A combination of Fe chelators and antioxidant treatment (e.g., N-acetylcysteine or ascorbic acid) has been proposed for myoglobin-induced AKI (Panizo et al., 2015). Nevertheless, Fe chelators are nephrotoxic, limiting their application (Kang et al., 2019).

Despite detailed knowledge of the *in vitro* molecular properties of chelating agents and antioxidants and their interactions with Cd (see Flora et al., 2008; Bjorklund et al., 2019), their clinical usefulness remains unproven apart from occasional case reports. Succimer, diethylenetriaminepentaacetate, and ethylenediaminetetraacetic acid (EDTA) have been considered as Cd chelating agents for acute Cd intoxication (reviewed in Smith, 2013). In animals, chelators can reduce acute Cd-induced mortality, provided treatment started very soon after Cd ingestion. Zn may exert protective effects against acute Cd toxicity that are partially MT-mediated, as suggested from neonatal murine-engineered cardiac tissues (Yu et al., 2020).

There is no recommended treatment for chronic Cd exposure in humans (reviewed in Smith, 2013), consistent with the Agency for Toxic Substances and Disease Registry (ATSDR) guidelines for Cd (Faroon et al., 2012). In particular, EDTA, was of no benefit in chronic Cd-induced renal dysfunction (Wu et al., 2004). This 14-year follow-up study of 17 patients was important because it challenges case reports suggesting beneficial effects of EDTA applied together with antioxidants, such as GSH (e.g., Gil et al., 2011). No recommended treatment strategy is available for Fe-induced CKD.

CONCLUSION AND OUTLOOK

Our review has summarized where the science and applications are currently for Fe and Cd entry into renal mitochondria listed in the sections of this review although we had to limit coverage. Still, despite wealth of current knowledge, several burning issues remain unresolved and require clarification in the near future. One hopes, for example, that biophysical methods will reveal Fe concentration and speciation for renal mitochondria. A largely unsolved issue is mitochondrial DMT1 import where several hypotheses concurrently prevail. DMT1 import could originate from the cytosol and involve a (co-translational) import pathway for mitochondrial proteins encoded in the nucleus. The "vesicular" hypothesis implies that DMT1 is targeted to the OMM through transient or permanent interactions with ER or endosomal vesicles.

Additional studies on isolated *b/b* vs. +/+ and +/*b* mitochondria should reveal whether other DMT1 substrates like Cd^{2+} or Cu^{1+} rely mostly on DMT1 to cross the OMM (as for Mn^{2+}) or have additional means for entry (like Fe^{2+}). The additional means for entry represents a more challenging issue because one must propose and test candidates where the design of the test could depend on the nature of the candidate. Also, finding the extent to which mitochondrial entry via DMT1 and "kiss and run" overlap also requires more hypothesis driven tests. Developing *in vitro* subcellular systems where components can come from different cells as a source could lead to future advances. An additional transport aspect concerns the entry pathways for Fe and Cd at the IMM. Direct proof of Mfrn1- and Mfrn2- dependent Fe transport at the IMM is missing, implying that other yet unknown pathways for Fe (and Cd) may be operative at the IMM as well. Although indirect evidence suggests Cd entry into the matrix via the MCU (Lee et al., 2005b), proof is lacking and would require a combination of molecular biology and electrophysiological approaches, as shown for Ca^{2+} (Kamer and Mootha, 2015; Mammucari et al., 2017).

Concerning the complicated, reciprocal relationship between (patho)physiological ROS and mitochondrial dynamics and plasticity, the role of fusion and fission events in ROS signaling is attracting much attention, postulating compartmentalization of damaging ROS through fission or antioxidant sharing through fusion. Moreover, how does formation of respiratory supercomplexes affect ROS production and balance between superoxide and H_2O_2? Changes in membrane lipids influence membrane-associated ROS-producing and ROS-metabolizing systems as well as the ETC and AQPs, therefore the impact of Fe/Cd on membrane lipid alterations affecting mitochondrial health warrants further investigation. Lastly, are mitochondria-derived physiological ROS signals involved in regulating Na^+/K^+-ATPase activity, which is indispensable for kidney function?

Fe overload and Cd accumulation occur in both kidney cortex and medulla (reviewed in Thévenod, 2010;

Thévenod and Wolff, 2016; Thévenod et al., 2019). But why is Fe and Cd nephrotoxicity less apparent in the distal nephron although transporters for these metal ions, receptors for metal-protein complexes and mitochondria prevail in these nephron segments? The relative resistance of the distal nephron to toxicity by these metal ions may result from its lower sensitivity to oxidative stress, an increased potential for adaptive responses and stress-induced factors (e.g., hypoxia-inducible factor-1α, hepcidin, and NGAL) (reviewed in Thévenod and Wolff, 2016), and its metabolic profile (see section "Nephron Transport and Metabolism: Focusing on Energy"). Indeed, all these issues could also account for the relative resistance of the kidney medulla to damage elicited by other inducers of AKI (Hall et al., 2013). A better understanding of differences in kidney cortex and medulla of transport, metabolism, mitochondrial dynamics, and stress/adaptive signaling may contribute to the development of preventive and novel therapeutic strategies for acute and chronic kidney injury induced by Cd and Fe.

AUTHOR CONTRIBUTIONS

FT received the invitation to writing this review who subsequently invited MG and W-KL to join. FT drafted sections "Introduction," "Nephron Transport and Metabolism: Focusing on Energy," "Mitochondrial Functions: Determinants of Life and Death," "Transport at OMM and IMM and Properties in Relation to Metal Ion Movement," "Fe Overload and Renal Injury," "Fe and Mitochondrial Damage," "Cd Toxicity and the Kidney," "Cd and Kidney Mitochondrial Damage," "Therapy," and **Figure 4**. MG drafted sections "Metal Distribution in Mitochondria – To What Purpose?," "A Recent, Versatile Candidate for OMM Import of Divalent Cations," "How Does DMT1 Integrate Into Mitochondrial Metal Homeostasis?," "Systemic and Cellular Iron Homeostasis," and **Figures 2**, **3**. W-KL drafted sections "ROS/RNS: Double-Edged Swords," "Redox Control Mechanisms Through Transport at Mitochondrial Membranes," and **Figure 1**. All authors drafted section "Conclusion and Outlook" and reviewed one another's drafts.

FUNDING

FT received funding from BMBF (01DN16039), DFG (TH345), and ZBAF. MG appreciates the support of grant R01 DK109717 from the National Institute of Diabetes and Digestive and Kidney Diseases and the Office of Dietary Supplements. W-KL received financial support through the Intramural Funding Program at Witten/Herdecke University (IFF 2018-52).

REFERENCES

Abouhamed, M., Gburek, J., Liu, W., Torchalski, B., Wilhelm, A., Wolff, N. A., et al. (2006). Divalent metal transporter 1 in the kidney proximal tubule is expressed in late endosomes/lysosomal membranes: implications for renal handling of protein-metal complexes. *Am. J. Physiol. Renal Physiol.* 290, F1525–F1533.

Abu-Hayyeh, S., Sian, M., Jones, K. G., Manuel, A., and Powell, J. T. (2001). Cadmium accumulation in aortas of smokers. *Arterioscler. Thromb. Vasc. Biol.* 21, 863–867. doi: 10.1161/01.atv.21.5.863

Adams, L., Franco, M. C., and Estevez, A. G. (2015). Reactive nitrogen species in cellular signaling. *Exp. Biol. Med.* 240, 711–717.

Alpern, R. J., Caplan, M. J., and Moe, O. W. (2013). *Seldin and Giebisch's The Kidney: Physiology & Pathophysiology*. London: Academic Press.

Antunes, F., and Cadenas, E. (2000). Estimation of H2O2 gradients across biomembranes. *FEBS Lett.* 475, 121–126. doi: 10.1016/s0014-5793(00)01638-0

Augustynek, B., Kunz, W. S., and Szewczyk, A. (2017). Guide to the Pharmacology of Mitochondrial Potassium Channels. *Handb Exp Pharmacol* 240, 103–127. doi: 10.1007/164_2016_79

Baines, C. P., and Gutierrez-Aguilar, M. (2018). The still uncertain identity of the channel-forming unit(s) of the mitochondrial permeability transition pore. *Cell Calcium* 73, 121–130. doi: 10.1016/j.ceca.2018.05.003

Baker, Z. N., Cobine, P. A., and Leary, S. C. (2017). The mitochondrion: a central architect of copper homeostasis. *Metallomics* 9, 1501–1512. doi: 10.1039/c7mt00221a

Basile, D. P., Anderson, M. D., and Sutton, T. A. (2012). Pathophysiology of acute kidney injury. *Compr. Physiol.* 2, 1303–1353.

Becker, T., and Wagner, R. (2018). Mitochondrial outer membrane channels: emerging diversity in transport processes. *Bioessays* 40:e1800013.

Bedard, K., and Krause, K. H. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* 87, 245–313. doi: 10.1152/physrev.00044.2005

Belyaeva, E. A., Glazunov, V. V., and Korotkov, S. M. (2002). Cyclosporin A-sensitive permeability transition pore is involved in Cd(2+)-induced dysfunction of isolated rat liver mitochondria: doubts no more. *Arch. Biochem. Biophys.* 405, 252–264. doi: 10.1016/s0003-9861(02)00400-9

Belyaeva, E. A., Glazunov, V. V., and Korotkov, S. M. (2004). Cd2+ -promoted mitochondrial permeability transition: a comparison with other heavy metals. *Acta Biochim. Pol.* 51, 545–551. doi: 10.18388/abp.2004_3590

Betten, R., Scharner, B., Probst, S., Edemir, B., Wolff, N. A., Langelueddecke, C., et al. (2018). Tonicity inversely modulates lipocalin-2 (Lcn2/24p3/NGAL) receptor (SLC22A17) and Lcn2 expression via Wnt/beta-catenin signaling in renal inner medullary collecting duct cells: implications for cell fate and bacterial infection. *Cell Commun. Signal.* 16:74.

Bienert, G. P., and Chaumont, F. (2014). Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim. Biophys. Acta* 1840, 1596–1604. doi: 10.1016/j.bbagen.2013.09.017

Bienert, G. P., Moller, A. L., Kristiansen, K. A., Schulz, A., Moller, I. M., Schjoerring, J. K., et al. (2007). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J. Biol. Chem.* 282, 1183–1192. doi: 10.1074/jbc.m603761200

Bienert, G. P., Schjoerring, J. K., and Jahn, T. P. (2006). Membrane transport of hydrogen peroxide. *Biochim. Biophys. Acta* 1758, 994–1003. doi: 10.1016/j.bbamem.2006.02.015

Billings, F. T. T., Ball, S. K., Roberts, L. J. II, and Pretorius, M. (2011). Postoperative acute kidney injury is associated with hemoglobinemia and an enhanced oxidative stress response. *Free Radic. Biol. Med.* 50, 1480–1487. doi: 10.1016/j.freeradbiomed.2011.02.011

Bjorklund, G., Crisponi, G., Nurchi, V. M., Cappai, R., Buha Djordjevic, A., and Aaseth, J. (2019). A review on coordination properties of Thiol-containing chelating agents towards mercury, cadmium, and lead. *Molecules* 24:3247. doi: 10.3390/molecules24183247

Block, K., Gorin, Y., and Abboud, H. E. (2009). Subcellular localization of Nox4 and regulation in diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14385–14390. doi: 10.1073/pnas.0906805106

Bonora, M., and Pinton, P. (2019). A new current for the mitochondrial permeability transition. *Trends Biochem. Sci.* 44, 559–561. doi: 10.1016/j.tibs.2019.04.009

Boulet, A., Vest, K. E., Maynard, M. K., Gammon, M. G., Russell, A. C., Mathews, A. T., et al. (2018). The mammalian phosphate carrier SLC25A3 is a mitochondrial copper transporter required for cytochrome c oxidase biogenesis. *J. Biol. Chem.* 293, 1887–1896. doi: 10.1074/jbc.ra117.000265

Bourdon, A., Minai, L., Serre, V., Jais, J.-P., Sarzi, E., Aubert, S., et al. (2007). Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. *Nat. Genet.* 39, 776–780. doi: 10.1038/ng2040

Brand, M. D. (2016). Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic. Biol. Med.* 100, 14–31. doi: 10.1016/j.freeradbiomed.2016.04.001

Brenner, B. M. (2008). *Brenner & Rector's The Kidney*. . Philadelphia, PA: Saunders Elsevier.

Bridges, C. C., and Zalups, R. K. (2005). Molecular and ionic mimicry and the transport of toxic metals. *Toxicol. Appl. Pharmacol.* 204, 274–308. doi: 10.1016/j.taap.2004.09.007

Brigelius-Flohe, R., and Flohe, L. (2011). Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid. Redox Signal.* 15, 2335–2381. doi: 10.1089/ars.2010.3534

Brissot, P., Troadec, M. B., Loreal, O., and Brissot, E. (2019). Pathophysiology and classification of iron overload diseases; update 2018. *Transfus Clin. Biol.* 26, 80–88. doi: 10.1016/j.tracli.2018.08.006

Brown, G. C., and Borutaite, V. (2008). Regulation of apoptosis by the redox state of cytochrome c. *Biochim. Biophys. Acta* 1777, 877–881. doi: 10.1016/j.bbabio.2008.03.024

Buvelot, H., Jaquet, V., and Krause, K. H. (2019). Mammalian NADPH Oxidases. *Methods Mol. Biol.* 1982, 17–36. doi: 10.1007/978-1-4939-9424-3_2

Cabantchik, Z. I. (2014). Labile iron in cells and body fluids: physiology, pathology, and pharmacology. *Front. Pharmacol.* 5:45. doi: 10.3389/fphar.2014.00045

Calamita, G., Ferri, D., Gena, P., Liquori, G. E., Cavalier, A., Thomas, D., et al. (2005). The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. *J. Biol. Chem.* 280, 17149–17153. doi: 10.1074/jbc.c400595200

Camaschella, C., Nai, A., and Silvestri, L. (2020). Iron metabolism and iron disorders revisited in the hepcidin era. *Haematologica* 105, 260–272. doi: 10.3324/haematol.2019.232124

Cameron, I., Mcnamee, P. M., Markham, A., Morgan, R. M., and Wood, M. (1986). The effects of cadmium on succinate and NADH-linked substrate oxidations in rat hepatic mitochondria. *J. Appl. Toxicol.* 6, 325–330. doi: 10.1002/jat.2550060505

Cavadini, P., Biasiotto, G., Poli, M., Levi, S., Verardi, R., Zanella, I., et al. (2007). RNA silencing of the mitochondrial ABCB7 transporter in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload. *Blood* 109, 3552–3559. doi: 10.1182/blood-2006-08-041632

Chen, C., and Paw, B. H. (2012). Cellular and mitochondrial iron homeostasis in vertebrates. *Biochim. Biophys. Acta* 1823, 1459–1467. doi: 10.1016/j.bbamcr.2012.01.003

Choong, G., Liu, Y., and Templeton, D. M. (2014). Interplay of calcium and cadmium in mediating cadmium toxicity. *Chem. Biol. Interact.* 211, 54–65. doi: 10.1016/j.cbi.2014.01.007

Christensen, E. I., and Birn, H. (2002). Megalin and cubilin: multifunctional endocytic receptors. *Nat. Rev. Mol. Cell Biol.* 3, 256–266.

Christenson, E. T., Gallegos, A. S., and Banerjee, A. (2018). In vitro reconstitution, functional dissection, and mutational analysis of metal ion transport by mitoferrin-1. *J. Biol. Chem.* 293, 3819–3828. doi: 10.1074/jbc.m117.817478

Clarkson, T. W. (1993). Molecular and ionic mimicry of toxic metals. *Annu. Rev. Pharmacol. Toxicol.* 33, 545–571. doi: 10.1146/annurev.pa.33.040193.002553

Colombini, M. (2016). The VDAC channel: Molecular basis for selectivity. *Biochim. Biophys. Acta* 1863, 2498–2502. doi: 10.1016/j.bbamcr.2016.01.019

Comte, J., Maisterrena, B., and Gautheron, D. C. (1976). Lipid composition and protein profiles of outer and inner membranes from pig heart mitochondria. Comparison with microsomes. *Biochim. Biophys. Acta* 419, 271–284. doi: 10.1016/0005-2736(76)90353-9

Cordeiro, R. M. (2014). Reactive oxygen species at phospholipid bilayers: distribution, mobility and permeation. *Biochim. Biophys. Acta* 1838, 438–444. doi: 10.1016/j.bbamem.2013.09.016

Cox, A. G., Winterbourn, C. C., and Hampton, M. B. (2009). Mitochondrial peroxiredoxin involvement in antioxidant defence and redox signalling. *Biochem. J.* 425, 313–325. doi: 10.1042/bj20091541

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., et al. (2010). Cadmium stress: an oxidative challenge. *Biometals* 23, 927–940.

Danielli, M., Marrone, J., Capiglioni, A. M., and Marinelli, R. A. (2019a). Data of H2O2 release from AQP8-knockdown rat hepatocyte mitochondria. *Data Brief.* 23:103722. doi: 10.1016/j.dib.2019.103722

Danielli, M., Marrone, J., Capiglioni, A. M., and Marinelli, R. A. (2019b). Mitochondrial aquaporin-8 is involved in SREBP-controlled hepatocyte cholesterol biosynthesis. *Free Radic. Biol. Med.* 131, 370–375. doi: 10.1016/j.freeradbiomed.2018.12.016

Das, A., Nag, S., Mason, A. B., and Barroso, M. M. (2016). Endosome–mitochondria interactions are modulated by iron release from transferrin. *J. Cell Biol.* 214, 831–845. doi: 10.1083/jcb.201602069

Dlouhy, A. C., and Outten, C. E. (2013). "The iron metallome in eukaryotic organisms," in *Metallomics and the Cell*, ed. L. Banci (Dordrecht: Springer), 241–278. doi: 10.1007/978-94-007-5561-1_8

Dorn, G. W. II (2019). Evolving concepts of mitochondrial dynamics. *Annu. Rev. Physiol.* 81, 1–17. doi: 10.1146/annurev-physiol-020518-114358

Dorta, D. J., Leite, S., Demarco, K. C., Prado, I. M., Rodrigues, T., Mingatto, F. E., et al. (2003). A proposed sequence of events for cadmium-induced mitochondrial impairment. *J. Inorg. Biochem.* 97, 251–257. doi: 10.1016/s0162-0134(03)00314-3

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* 82, 47–95. doi: 10.1152/physrev.00018.2001

Faroon, O., Ashizawa, A., Wright, S., Tucker, P., Jenkins, K., Ingerman, L., et al. (2012). *Toxicological Profile for Cadmium*. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Ferguson, C. J., Wareing, M., Ward, D. T., Green, R., Smith, C. P., and Riccardi, D. (2001). Cellular localization of divalent metal transporter DMT-1 in rat kidney. *Am. J. Physiol. Renal Physiol.* 280, F803–F814.

Fernie, A. R., Carrari, F., and Sweetlove, L. J. (2004). Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Curr. Opin. Plant Biol.* 7, 254–261. doi: 10.1016/j.pbi.2004.03.007

Flora, S. J., Mittal, M., and Mehta, A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J. Med. Res.* 128, 501–523.

Fotiadis, D., Suda, K., Tittmann, P., Jeno, P., Philippsen, A., Muller, D. J., et al. (2002). Identification and structure of a putative Ca2+-binding domain at the C terminus of AQP1. *J. Mol. Biol.* 318, 1381–1394. doi: 10.1016/s0022-2836(02)00143-2

Freisinger, E., and Vasak, M. (2013). Cadmium in metallothioneins. *Met. Ions Life Sci.* 11, 339–371. doi: 10.1007/978-94-007-5179-8_11

Fukai, T., and Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid. Redox Signal.* 15, 1583–1606. doi: 10.1089/ars.2011.3999

Funk, J. A., and Schnellmann, R. G. (2012). Persistent disruption of mitochondrial homeostasis after acute kidney injury. *Am. J. Physiol. Renal Physiol.* 302, F853–F864.

Galleano, M., Farre, S. M., Turrens, J. F., and Puntarulo, S. (1994). Resistance of rat kidney mitochondrial membranes to oxidation induced by acute iron overload. *Toxicology* 88, 141–149. doi: 10.1016/0300-483x(94)90116-3

Garrick, M. D. (2016). "Regulation of DMT1 expression and function," in *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals*, ed. J. F. Collins (Amsterdam: Elsevier), 227–238.

Garrick, M. D., Kuo, H.-C., Vargas, F., Singleton, S., Zhao, L., Smith, J. J., et al. (2006a). Comparison of mammalian cell lines expressing distinct isoforms of divalent metal transporter 1 in a tetracycline-regulated fashion. *Biochem. J.* 398, 539–546. doi: 10.1042/bj20051987

Garrick, M. D., Singleton, S. T., Vargas, F., Kuo, H., Zhao, L., Knöpfel, M., et al. (2006b). DMT1: Which metals does it transport? *Biol. Res.* 39, 79–85.

Garrick, M. and Thévenod, F. (2019). *Getting metals into mitochondria. Atlas of Science*. Available online at: https://atlasofscience.org/getting-metals-into-mitochondria/ (accessed February 26, 2019).

Gburek, J., Verroust, P. J., Willnow, T. E., Fyfe, J. C., Nowacki, W., Jacobsen, C., et al. (2002). Megalin and cubilin are endocytic receptors involved in renal clearance of hemoglobin. *J. Am. Soc. Nephrol.* 13, 423–430.

Ghio, A. J., Soukup, J. M., Dailey, L. A., Richards, J. H., and Tong, H. (2016). The biological effect of asbestos exposure is dependent on changes in iron homeostasis. *Inhal. Toxicol.* 28, 698–705. doi: 10.1080/08958378.2016.1257665

Ghio, A. J., Tong, H., Soukup, J. M., Dailey, L. A., Cheng, W.-Y., Samet, J. M., et al. (2013). Sequestration of mitochondrial iron by silica particle initiates a biological effect. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 305, L712–L724.

Gibb, H. J., Barchowsky, A., Bellinger, D., Bolger, P. M., Carrington, C., Havelaar, A. H., et al. (2019). Estimates of the 2015 global and regional disease burden from four foodborne metals - arsenic, cadmium, lead and methylmercury. *Environ. Res.* 174, 188–194. doi: 10.1016/j.envres.2018.12.062

Gil, H. W., Kang, E. J., Lee, K. H., Yang, J. O., Lee, E. Y., and Hong, S. Y. (2011). Effect of glutathione on the cadmium chelation of EDTA in a patient with cadmium intoxication. *Hum. Exp. Toxicol.* 30, 79–83. doi: 10.1177/0960327110369818

Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2009). Mitochondria as targets for cancer chemotherapy. *Semin. Cancer Biol.* 19, 57–66. doi: 10.1016/j.semcancer.2008.11.007

Guder, W. G., and Ross, B. D. (1984). Enzyme distribution along the nephron. *Kidney Int.* 26, 101–111. doi: 10.1038/ki.1984.143

Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., et al. (1997). Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388, 482–488. doi: 10.1038/41343

Hall, A. M., Rhodes, G. J., Sandoval, R. M., Corridon, P. R., and Molitoris, B. A. (2013). In vivo multiphoton imaging of mitochondrial structure and function during acute kidney injury. *Kidney Int.* 83, 72–83. doi: 10.1038/ki.2012.328

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am. J. Med.* 91, 14S–22S.

Hamdi, A., Roshan, T. M., Kahawita, T. M., Mason, A. B., Sheftel, A. D., and Ponka, P. (2016). Erythroid cell mitochondria receive endosomal iron by a "kiss-and-run" mechanism. *Biochim. Biophys. Acta* 1863, 2859–2867. doi: 10.1016/j.bbamcr.2016.09.008

Han, D., Antunes, F., Canali, R., Rettori, D., and Cadenas, E. (2003). Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J. Biol. Chem.* 278, 5557–5563. doi: 10.1074/jbc.m210269200

Handy, D. E., Lubos, E., Yang, Y., Galbraith, J. D., Kelly, N., Zhang, Y. Y., et al. (2009). Glutathione peroxidase-1 regulates mitochondrial function to modulate redox-dependent cellular responses. *J. Biol. Chem.* 284, 11913–11921. doi: 10.1074/jbc.m900392200

Hartwig, A. (2013). Cadmium and cancer. *Met. Ions Life Sci.* 11, 491–507.

Hawkins, B. J., Madesh, M., Kirkpatrick, C. J., and Fisher, A. B. (2007). Superoxide flux in endothelial cells via the chloride channel-3 mediates intracellular signaling. *Mol. Biol. Cell* 18, 2002–2012. doi: 10.1091/mbc.e06-09-0830

Hayyan, M., Hashim, M. A., and Alnashef, I. M. (2016). Superoxide ion: generation and chemical implications. *Chem. Rev.* 116, 3029–3085. doi: 10.1021/acs.chemrev.5b00407

Hentze, M. W., Muckenthaler, M. U., Galy, B., and Camaschella, C. (2010). Two to tango: regulation of Mammalian iron metabolism. *Cell* 142, 24–38. doi: 10.1016/j.cell.2010.06.028

Herrmann, J. M., and Riemer, J. (2010). The intermembrane space of mitochondria. *Antioxid. Redox Signal.* 13, 1341–1358. doi: 10.1089/ars.2009.3063

Holley, A. K., Bakthavatchalu, V., Velez-Roman, J. M., and St Clair, D. K. (2011). Manganese superoxide dismutase: guardian of the powerhouse. *Int. J. Mol. Sci.* 12, 7114–7162. doi: 10.3390/ijms12107114

Holmes-Hampton, G. P., Jhurry, N. D., Mccormick, S. P., and Lindahl, P. A. (2013). Iron content of Saccharomyces cerevisiae cells grown under iron-deficient and iron-overload conditions. *Biochemistry* 52, 105–114. doi: 10.1021/bi3015339

Holmstrom, K. M., and Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat. Rev. Mol. Cell Biol.* 15, 411–421. doi: 10.1038/nrm3801

Houamel, D., Ducrot, N., Lefebvre, T., Daher, R., Moulouel, B., Sari, M. A., et al. (2016). Hepcidin as a major component of renal antibacterial defenses against uropathogenic *Escherichia coli*. *J. Am. Soc. Nephrol.* 27, 835–846. doi: 10.1681/asn.2014101035

Huff, J., Lunn, R. M., Waalkes, M. P., Tomatis, L., and Infante, P. F. (2007). Cadmium-induced cancers in animals and in humans. *Int. J. Occup. Environ. Health* 13, 202–212. doi: 10.1179/oeh.2007.13.2.202

Illing, A. C., Shawki, A., Cunningham, C. L., and Mackenzie, B. (2012). Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. *J. Biol. Chem.* 287, 30485–30496. doi: 10.1074/jbc.m112.364208

Jagetia, G. C., and Reddy, T. K. (2011). Alleviation of iron induced oxidative stress by the grape fruit flavanone naringin in vitro. *Chem. Biol. Interact.* 190, 121–128. doi: 10.1016/j.cbi.2011.02.009

Jarup, L., and Akesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.* 238, 201–208. doi: 10.1016/j.taap.2009.04.020

Jonckheere, A. I., Smeitink, J. A., and Rodenburg, R. J. (2012). Mitochondrial ATP synthase: architecture, function and pathology. *J. Inherit. Metab. Dis.* 35, 211–225. doi: 10.1007/s10545-011-9382-9

Kaludercic, N., Deshwal, S., and Di Lisa, F. (2014). Reactive oxygen species and redox compartmentalization. *Front. Physiol.* 5:285. doi: 10.3389/fphys.2014.00285

Kalyanaraman, B., Cheng, G., Hardy, M., Ouari, O., Bennett, B., and Zielonka, J. (2018). Teaching the basics of reactive oxygen species and their relevance to cancer biology: mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies. *Redox Biol.* 15, 347–362. doi: 10.1016/j.redox.2017.12.012

Kamer, K. J., and Mootha, V. K. (2015). The molecular era of the mitochondrial calcium uniporter. *Nat. Rev. Mol. Cell Biol.* 16, 545–553. doi: 10.1038/nrm4039

Kang, H., Han, M., Xue, J., Baek, Y., Chang, J., Hu, S., et al. (2019). Renal clearable nanochelators for iron overload therapy. *Nat. Commun.* 10:5134.

Karnati, S., Luers, G., Pfreimer, S., and Baumgart-Vogt, E. (2013). Mammalian SOD2 is exclusively located in mitochondria and not present in peroxisomes. *Histochemie* 140, 105–117. doi: 10.1007/s00418-013-1099-4

Katz, A. I. (1982). Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am. J. Physiol.* 242, F207–F219.

Katz, A. I., Doucet, A., and Morel, F. (1979). Na-K-ATPase activity along the rabbit, rat, and mouse nephron. *Am. J. Physiol.* 237, F114–F120.

Kerek, E. M., and Prenner, E. J. (2016). Inorganic cadmium affects the fluidity and size of phospholipid based liposomes. *Biochim. Biophys. Acta* 1858, 3169–3181. doi: 10.1016/j.bbamem.2016.10.005

Kirichok, Y., Krapivinsky, G., and Clapham, D. E. (2004). The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 427, 360–364. doi: 10.1038/nature02246

Klusch, N., Murphy, B. J., Mills, D. J., Yildiz, O., and Kuhlbrandt, W. (2017). Structural basis of proton translocation and force generation in mitochondrial ATP synthase. *eLife* 6:e33274.

Kojer, K., Bien, M., Gangel, H., Morgan, B., Dick, T. P., and Riemer, J. (2012). Glutathione redox potential in the mitochondrial intermembrane space is linked to the cytosol and impacts the Mia40 redox state. *EMBO J.* 31, 3169–3182. doi: 10.1038/emboj.2012.165

Kozyraki, R., Fyfe, J., Verroust, P. J., Jacobsen, C., Dautry-Varsat, A., Gburek, J., et al. (2001). Megalin-dependent cubilin-mediated endocytosis is a major pathway for the apical uptake of transferrin in polarized epithelia. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12491–12496. doi: 10.1073/pnas.211291398

Kroemer, G., Galluzzi, L., and Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* 87, 99–163. doi: 10.1152/physrev.00013.2006

Kuksal, N., Chalker, J., and Mailloux, R. J. (2017). Progress in understanding the molecular oxygen paradox - function of mitochondrial reactive oxygen species in cell signaling. *Biol. Chem.* 398, 1209–1227. doi: 10.1515/hsz-2017-0160

Lane, D. J., Merlot, A. M., Huang, M. L., Bae, D. H., Jansson, P. J., Sahni, S., et al. (2015). Cellular iron uptake, trafficking and metabolism: key molecules and mechanisms and their roles in disease. *Biochim. Biophys. Acta* 1853, 1130–1144. doi: 10.1016/j.bbamcr.2015.01.021

Langelueddecke, C., Roussa, E., Fenton, R. A., Wolff, N. A., Lee, W. K., and Thévenod, F. (2012). Lipocalin-2 (24p3/neutrophil gelatinase-associated lipocalin (NGAL)) receptor is expressed in distal nephron and mediates protein endocytosis. *J. Biol. Chem.* 287, 159–169. doi: 10.1074/jbc.m111.308296

Lee, W. K., Bork, U., Gholamrezaei, F., and Thévenod, F. (2005a). Cd(2+)-induced cytochrome c release in apoptotic proximal tubule cells: role of mitochondrial permeability transition pore and Ca(2+) uniporter. *Am. J. Physiol. Renal Physiol.* 288, F27–F39.

Lee, W. K., Spielmann, M., Bork, U., and Thévenod, F. (2005b). Cd2+-induced swelling-contraction dynamics in isolated kidney cortex mitochondria: role of Ca2+ uniporter, K+ cycling, and protonmotive force. *Am. J. Physiol. Cell Physiol.* 289, C656–C664.

Lee, W. K., and Thévenod, F. (2006). A role for mitochondrial aquaporins in cellular life-and-death decisions? *Am. J. Physiol. Cell Physiol.* 291, C195–C202.

Lee, W. K., and Thévenod, F. (2020). Cell organelles as targets of mammalian cadmium toxicity. *Arch. Toxicol.* 94, 1017–1049. doi: 10.1007/s00204-020-02692-8

Letts, J. A., and Sazanov, L. A. (2017). Clarifying the supercomplex: the higher-order organization of the mitochondrial electron transport chain. *Nat. Struct. Mol. Biol.* 24, 800–808. doi: 10.1038/nsmb.3460

Li, M., Xia, T., Jiang, C. S., Li, L. J., Fu, J. L., and Zhou, Z. C. (2003). Cadmium directly induced the opening of membrane permeability pore of mitochondria which possibly involved in cadmium-triggered apoptosis. *Toxicology* 194, 19–33. doi: 10.1016/s0300-483x(03)00327-5

Lill, R., Hoffmann, B., Molik, S., Pierik, A. J., Rietzschel, N., Stehling, O., et al. (2012). The role of mitochondria in cellular iron–sulfur protein biogenesis and iron metabolism. *Biochim. Biophys. Acta* 1823, 1491–1508. doi: 10.1016/j.bbamcr.2012.05.009

Lim, J. B., Huang, B. K., Deen, W. M., and Sikes, H. D. (2015). Analysis of the lifetime and spatial localization of hydrogen peroxide generated in the cytosol using a reduced kinetic model. *Free Radic. Biol. Med.* 89, 47–53. doi: 10.1016/j.freeradbiomed.2015.07.009

Lipper, C. H., Stofleth, J. T., Bai, F., Sohn, Y. S., Roy, S., Mittler, R., et al. (2019). Redox-dependent gating of VDAC by mitoNEET. *Proc. Natl. Acad. Sci. U.S.A.* 116, 19924–19929. doi: 10.1073/pnas.1908271116

Liu, B. C., Tang, T. T., Lv, L. L., and Lan, H. Y. (2018). Renal tubule injury: a driving force toward chronic kidney disease. *Kidney Int.* 93, 568–579. doi: 10.1016/j.kint.2017.09.033

Lizana, L., Bauer, B., and Orwar, O. (2008). Controlling the rates of biochemical reactions and signaling networks by shape and volume changes. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4099–4104. doi: 10.1073/pnas.0709932105

Mackova, K., Misak, A., and Tomaskova, Z. (2018). "Lifting the fog over mitochondrial chloride channels," in *Ion Channels in Health and Sickness*, ed. K. F. Shad (London: IntechOpen Limited).

Madesh, M., and Hajnoczky, G. (2001). VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. *J. Cell Biol.* 155, 1003–1015. doi: 10.1083/jcb.200105057

Mailloux, R. J. (2018). Mitochondrial antioxidants and the maintenance of cellular hydrogen peroxide levels. *Oxid. Med. Cell. Longev.* 2018:7857251.

Mammucari, C., Gherardi, G., and Rizzuto, R. (2017). Structure, activity regulation, and role of the mitochondrial calcium uniporter in health and disease. *Front. Oncol.* 7:139. doi: 10.3389/fonc.2017.00139

Marcus, Y. (1988). Ionic radii in aqueous solutions. *Chem. Rev.* 88, 1475–1498. doi: 10.1021/cr00090a003

Maret, W., and Moulis, J. M. (2013). The bioinorganic chemistry of cadmium in the context of its toxicity. *Met. Ions Life Sci.* 11, 1–29. doi: 10.1007/978-94-007-5179-8_1

Mari, M., Morales, A., Colell, A., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2009). Mitochondrial glutathione, a key survival antioxidant. *Antioxid. Redox Signal.* 11, 2685–2700. doi: 10.1089/ars.2009.2695

Marinho, H. S., Real, C., Cyrne, L., Soares, H., and Antunes, F. (2014). Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol.* 2, 535–562. doi: 10.1016/j.redox.2014.02.006

Martin, F. M., Xu, X., Von Lohneysen, K., Gilmartin, T. J., and Friedman, J. S. (2011). SOD2 deficient erythroid cells up-regulate transferrin receptor and down-regulate mitochondrial biogenesis and metabolism. *PLoS One* 6:e16894. doi: 10.1371/journal.pone.0016894

Maynard, L. S., and Cotzias, G. C. (1955). The partition of manganese among organs and intracellular organelles of the rat. *J. Biol. Chem.* 214, 489–495.

Mccommis, K. S., and Finck, B. N. (2015). Mitochondrial pyruvate transport: a historical perspective and future research directions. *Biochem. J.* 466, 443–454. doi: 10.1042/bj20141171

Mckernan, T. B., Woods, E. B., and Lash, L. H. (1991). Uptake of glutathione by renal cortical mitochondria. *Arch. Biochem. Biophys.* 288, 653–663. doi: 10.1016/0003-9861(91)90248-h

Mitchell, C. J., Shawki, A., Ganz, T., Nemeth, E., and Mackenzie, B. (2014). Functional properties of human ferroportin, a cellular iron exporter reactive also with cobalt and zinc. *Am. J. Physiol. Cell Physiol.* 306, C450–C459.

Moulis, J. M. (2010). Cellular mechanisms of cadmium toxicity related to the homeostasis of essential metals. *Biometals* 23, 877–896. doi: 10.1007/s10534-010-9336-y

Moulis, J. M., and Thévenod, F. (2010). New perspectives in cadmium toxicity: an introduction. *Biometals* 23, 763–768. doi: 10.1007/s10534-010-9365-6

Moulouel, B., Houamel, D., Delaby, C., Tchernitchko, D., Vaulont, S., Letteron, P., et al. (2013). Hepcidin regulates intrarenal iron handling at the distal nephron. *Kidney Int.* 84, 756–766. doi: 10.1038/ki.2013.142

Munro, D., and Treberg, J. R. (2017). A radical shift in perspective: mitochondria as regulators of reactive oxygen species. *J. Exp. Biol.* 220, 1170–1180. doi: 10.1242/jeb.132142

Musatov, A., and Robinson, N. C. (2012). Susceptibility of mitochondrial electron-transport complexes to oxidative damage. Focus on cytochrome c oxidase. *Free Radic. Res.* 46, 1313–1326. doi: 10.3109/10715762.2012.717273

Nair, A. R., Lee, W. K., Smeets, K., Swennen, Q., Sanchez, A., Thévenod, F., et al. (2015). Glutathione and mitochondria determine acute defense responses and adaptive processes in cadmium-induced oxidative stress and toxicity of the kidney. *Arch. Toxicol.* 89, 2273–2289. doi: 10.1007/s00204-014-1401-9

Nakanishi, T., Kuragano, T., Nanami, M., Nagasawa, Y., and Hasuike, Y. (2019). Misdistribution of iron and oxidative stress in chronic kidney disease. *Free Radic. Biol. Med.* 133, 248–253. doi: 10.1016/j.freeradbiomed.2018.06.025

Nawrot, T. S., Staessen, J. A., Roels, H. A., Munters, E., Cuypers, A., Richart, T., et al. (2010). Cadmium exposure in the population: from health risks to strategies of prevention. *Biometals* 23, 769–782. doi: 10.1007/s10534-010-9343-z

Nisimoto, Y., Diebold, B. A., Cosentino-Gomes, D., and Lambeth, J. D. (2014). Nox4: a hydrogen peroxide-generating oxygen sensor. *Biochemistry* 53, 5111–5120. doi: 10.1021/bi500331y

Nowikovsky, K., Schweyen, R. J., and Bernardi, P. (2009). Pathophysiology of mitochondrial volume homeostasis: potassium transport and permeability transition. *Biochim. Biophys. Acta* 1787, 345–350. doi: 10.1016/j.bbabio.2008.10.006

Ott, M., Robertson, J. D., Gogvadze, V., Zhivotovsky, B., and Orrenius, S. (2002). Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1259–1263. doi: 10.1073/pnas.241655498

Palmieri, F., and Monne, M. (2016). Discoveries, metabolic roles and diseases of mitochondrial carriers: a review. *Biochim. Biophys. Acta* 1863, 2362–2378. doi: 10.1016/j.bbamcr.2016.03.007

Pan, J., Plant, J. A., Voulvoulis, N., Oates, C. J., and Ihlenfeld, C. (2010). Cadmium levels in Europe: implications for human health. *Environ. Geochem. Health* 32, 1–12. doi: 10.1007/s10653-009-9273-2

Panizo, N., Rubio-Navarro, A., Amaro-Villalobos, J. M., Egido, J., and Moreno, J. A. (2015). Molecular mechanisms and novel therapeutic approaches to rhabdomyolysis-induced acute kidney injury. *Kidney Blood Press Res.* 40, 520–532. doi: 10.1159/000368528

Papanikolaou, G., and Pantopoulos, K. (2017). Systemic iron homeostasis and erythropoiesis. *IUBMB Life* 69, 399–413. doi: 10.1002/iub.1629

Paradkar, P. N., Zumbrennen, K. B., Paw, B. H., Ward, D. M., and Kaplan, J. (2009). Regulation of mitochondrial iron import through differential turnover of mitoferrin 1 and mitoferrin 2. *Mol. Cell. Biol.* 29, 1007–1016. doi: 10.1128/mcb.01685-08

Parajuli, N., Marine, A., Simmons, S., Saba, H., Mitchell, T., Shimizu, T., et al. (2011). Generation and characterization of a novel kidney-specific manganese superoxide dismutase knockout mouse. *Free Radic. Biol. Med.* 51, 406–416. doi: 10.1016/j.freeradbiomed.2011.04.024

Pastore, A., and Puccio, H. (2013). Frataxin: a protein in search for a function. *J. Neurochem.* 126(Suppl. 1), 43–52. doi: 10.1111/jnc.12220

Patterson, H. C., Gerbeth, C., Thiru, P., Vogtle, N. F., Knoll, M., Shahsafaei, A., et al. (2015). A respiratory chain controlled signal transduction cascade in the mitochondrial intermembrane space mediates hydrogen peroxide signaling. *Proc. Natl. Acad. Sci. U.S.A.* 112, E5679–E5688.

Paul, B. T., Manz, D. H., Torti, F. M., and Torti, S. V. (2017). Mitochondria and Iron: current questions. *Expert Rev. Hematol.* 10, 65–79. doi: 10.1080/17474086.2016.1268047

Petering, D. H. (2017). Reactions of the Zn Proteome with Cd(2+) and Other Xenobiotics: trafficking and Toxicity. *Chem. Res. Toxicol.* 30, 189–202. doi: 10.1021/acs.chemrestox.6b00328

Petrosillo, G., Ruggiero, F. M., and Paradies, G. (2003). Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. *FASEB J.* 17, 2202–2208. doi: 10.1096/fj.03-0012com

Pfaller, W. (1982). Structure function correlation on rat kidney. Quantitative correlation of structure and function in the normal and injured rat kidney. *Adv. Anat. Embryol. Cell Biol.* 70, 1–106. doi: 10.1007/978-3-642-68287-2_1

Pfanner, N., Warscheid, B., and Wiedemann, N. (2019). Mitochondrial proteins: from biogenesis to functional networks. *Nat. Rev. Mol. Cell Biol.* 20, 267–284. doi: 10.1038/s41580-018-0092-0

Philpott, C. C., and Ryu, M. S. (2014). Special delivery: distributing iron in the cytosol of mammalian cells. *Front. Pharmacol.* 5:173. doi: 10.3389/fphar.2014.00173

Pierrel, F., Cobine, P. A., and Winge, D. R. (2007). Metal Ion availability in mitochondria. *BioMetals* 20, 675–682. doi: 10.1007/s10534-006-9052-9

Plotnikov, E. Y., Chupyrkina, A. A., Pevzner, I. B., Isaev, N. K., and Zorov, D. B. (2009). Myoglobin causes oxidative stress, increase of NO production and dysfunction of kidney's mitochondria. *Biochim. Biophys. Acta* 1792, 796–803. doi: 10.1016/j.bbadis.2009.06.005

Porcelli, A. M., Ghelli, A., Zanna, C., Pinton, P., Rizzuto, R., and Rugolo, M. (2005). pH difference across the outer mitochondrial membrane measured with a green fluorescent protein mutant. *Biochem. Biophys. Res. Commun.* 326, 799–804. doi: 10.1016/j.bbrc.2004.11.105

Quinlan, C. L., Goncalves, R. L., Hey-Mogensen, M., Yadava, N., Bunik, V. I., and Brand, M. D. (2014). The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I. *J. Biol. Chem.* 289, 8312–8325. doi: 10.1074/jbc.m113.545301

Ray, P. D., Huang, B. W., and Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal.* 24, 981–990. doi: 10.1016/j.cellsig.2012.01.008

Ribas, V., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2014). Glutathione and mitochondria. *Front. Pharmacol.* 5:151. doi: 10.3389/fphar.2014.00151

Richardson, D. R., Lane, D. J., Becker, E. M., Huang, M. L., Whitnall, M., Suryo Rahmanto, Y., et al. (2010). Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10775–10782. doi: 10.1073/pnas.0912925107

Rieger, B., Junge, W., and Busch, K. B. (2014). Lateral pH gradient between OXPHOS complex IV and F(0)F(1) ATP-synthase in folded mitochondrial membranes. *Nat. Commun.* 5:3103.

Robb, E. L., Hall, A. R., Prime, T. A., Eaton, S., Szibor, M., Viscomi, C., et al. (2018). Control of mitochondrial superoxide production by reverse electron transport at complex I. *J. Biol. Chem.* 293, 9869–9879. doi: 10.1074/jbc.ra118.003647

Roth, J. A., Singleton, S., Feng, J., Garrick, M., and Paradkar, P. N. (2010). Parkin regulates metal transport via proteasomal degradation of the 1B isoforms of divalent metal transporter 1. *J. Neurochem.* 113, 454–464. doi: 10.1111/j.1471-4159.2010.06607.x

Sabharwal, S. S., Waypa, G. B., Marks, J. D., and Schumacker, P. T. (2013). Peroxiredoxin-5 targeted to the mitochondrial intermembrane space attenuates hypoxia-induced reactive oxygen species signalling. *Biochem. J.* 456, 337–346. doi: 10.1042/bj20130740

Sakurai, H., Nishida, M., Yoshimura, T., Takada, J., and Koyama, M. (1985). Partition of divalent and total manganese in organs and subcellular organelles of MnCl2-treated rats studied by ESR and neutron activation analysis. *Biochim. Biophys. Acta* 841, 208–214. doi: 10.1016/0304-4165(85)90023-6

Salmela, S. S., Vuori, E., Huunan-Seppala, A., Kilpio, J. O., and Sumuvuori, H. (1983). Body burden of cadmium in man at low level of exposure. *Sci. Total Environ.* 27, 89–95. doi: 10.1016/0048-9697(83)90126-2

Satarug, S., Gobe, G. C., Ujjin, P., and Vesey, D. A. (2020). A comparison of the nephrotoxicity of low doses of cadmium and lead. *Toxics* 8:18. doi: 10.3390/toxics8010018

Satarug, S., and Moore, M. R. (2004). Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environ. Health Perspect.* 112, 1099–1103. doi: 10.1289/ehp.6751

Scarpulla, R. C. (2012). Nucleus-encoded regulators of mitochondrial function: integration of respiratory chain expression, nutrient sensing and metabolic stress. *Biochim. Biophys. Acta* 1819, 1088–1097. doi: 10.1016/j.bbagrm.2011.10.011

Schmidt, U., and Guder, W. G. (1976). Sites of enzyme activity along the nephron. *Kidney Int.* 9, 233–242. doi: 10.1038/ki.1976.26

Schuh, C. D., Polesel, M., Platonova, E., Haenni, D., Gassama, A., Tokonami, N., et al. (2018). Combined structural and functional imaging of the kidney reveals major axial differences in proximal tubule endocytosis. *J. Am. Soc. Nephrol.* 29, 2696–2712.

Scindia, Y., Dey, P., Thirunagari, A., Liping, H., Rosin, D. L., Floris, M., et al. (2015). Hepcidin mitigates renal ischemia-reperfusion injury by modulating systemic iron homeostasis. *J. Am. Soc. Nephrol.* 26, 2800–2814. doi: 10.1681/asn.2014101037

Shadel, G. S., and Horvath, T. L. (2015). Mitochondrial ROS signaling in organismal homeostasis. *Cell* 163, 560–569. doi: 10.1016/j.cell.2015.10.001

Shanmugasundaram, K., Nayak, B. K., Friedrichs, W. E., Kaushik, D., Rodriguez, R., and Block, K. (2017). NOX4 functions as a mitochondrial energetic sensor coupling cancer metabolic reprogramming to drug resistance. *Nat. Commun.* 8:997.

Sharma, S., and Leaf, D. E. (2019). Iron chelation as a potential therapeutic strategy for AKI prevention. *J. Am. Soc. Nephrol.* 30, 2060–2071. doi: 10.1681/asn.2019060595

Sheftel, A. D., Zhang, A. S., Brown, C., Shirihai, O. S., and Ponka, P. (2007). Direct interorganellar transfer of iron from endosome to mitochondrion. *Blood* 110, 125–132. doi: 10.1182/blood-2007-01-068148

Sies, H., and Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21, 363–383. doi: 10.1038/s41580-020-0230-3

Slotki, I., and Cabantchik, Z. I. (2015). The labile side of iron supplementation in CKD. *J. Am. Soc. Nephrol.* 26, 2612–2619. doi: 10.1681/asn.2015010052

Smith, C. P., Lee, W. K., Haley, M., Poulsen, S. B., Thévenod, F., and Fenton, R. A. (2019). Proximal tubule transferrin uptake is modulated by cellular iron and mediated by apical membrane megalin-cubilin complex and transferrin receptor 1. *J. Biol. Chem.* 294, 7025–7036.

Smith, C. P., and Thévenod, F. (2009). Iron transport and the kidney. *Biochim. Biophys. Acta* 1790, 724–730.

Smith, S. W. (2013). The role of chelation in the treatment of other metal poisonings. *J. Med. Toxicol.* 9, 355–369. doi: 10.1007/s13181-013-0343-6

Swaminathan, S. (2018). Iron homeostasis pathways as therapeutic targets in acute kidney injury. *Nephron* 140, 156–159. doi: 10.1159/000490808

Szabo, I., Leanza, L., Gulbins, E., and Zoratti, M. (2012). Physiology of potassium channels in the inner membrane of mitochondria. *Pflugers Arch.* 463, 231–246. doi: 10.1007/s00424-011-1058-7

Tan, W., and Colombini, M. (2007). VDAC closure increases calcium ion flux. *Biochim. Biophys. Acta* 1768, 2510–2515. doi: 10.1016/j.bbamem.2007.06.002

Thévenod, F. (2009). Cadmium and cellular signaling cascades: To be or not to be? *Toxicol. Appl. Pharmacol.* 238, 221–239. doi: 10.1016/j.taap.2009.01.013

Thévenod, F. (2010). Catch me if you can! Novel aspects of cadmium transport in mammalian cells. *Biometals* 23, 857–875. doi: 10.1007/s10534-010-9309-1

Thévenod, F., Fels, J., Lee, W. K., and Zarbock, R. (2019). Channels, transporters and receptors for cadmium and cadmium complexes in eukaryotic cells: myths and facts. *Biometals* 32, 469–489. doi: 10.1007/s10534-019-00176-6

Thévenod, F., and Lee, W. K. (2013). Cadmium and cellular signaling cascades: interactions between cell death and survival pathways. *Arch. Toxicol.* 87, 1743–1786. doi: 10.1007/s00204-013-1110-9

Thévenod, F., and Wolff, N. A. (2016). Iron transport in the kidney: implications for physiology and cadmium nephrotoxicity. *Metallomics* 8, 17–42. doi: 10.1039/c5mt00215j

Tong, J., Briggs, M. M., and Mcintosh, T. J. (2012). Water permeability of aquaporin-4 channel depends on bilayer composition, thickness, and elasticity. *Biophys. J.* 103, 1899–1908. doi: 10.1016/j.bpj.2012.09.025

Valdez, L. B., Bombicino, S. S., Iglesias, D. E., Mikusic, I. A. R., and Boveris, A. (2018). Mitochondrial peroxynitrite generation is mainly driven by superoxide steady-state concentration rather than by nitric oxide steady-state concentration. *Int J. Mol. Biol.* 3, 56–61.

Van Swelm, R. P. L., Vos, M., Verhoeven, F., Thévenod, F., and Swinkels, D. W. (2018). Endogenous hepcidin synthesis protects the distal nephron against hemin and hemoglobin mediated necroptosis. *Cell Death Dis.* 9:550.

Van Swelm, R. P. L., Wetzels, J. F. M., and Swinkels, D. W. (2020). The multifaceted role of iron in renal health and disease. *Nat. Rev. Nephrol.* 16, 77–98. doi: 10.1038/s41581-019-0197-5

Veal, E. A., Day, A. M., and Morgan, B. A. (2007). Hydrogen peroxide sensing and signaling. *Mol. Cell* 26, 1–14. doi: 10.1016/j.molcel.2007.03.016

Wang, H., Schoebel, S., Schmitz, F., Dong, H., and Hedfalk, K. (2020). Characterization of aquaporin-driven hydrogen peroxide transport. *Biochim. Biophys. Acta Biomembr.* 1862, 183065. doi: 10.1016/j.bbamem.2019.183065

Wang, X., Garrick, M. D., and Collins, J. F. (2019). Animal models of normal and disturbed iron and copper metabolism. *J. Nutr.* 149, 2085–2100. doi: 10.1093/jn/nxz172

Wang, Y., Fang, J., Leonard, S. S., and Rao, K. M. (2004). Cadmium inhibits the electron transfer chain and induces reactive oxygen species. *Free Radic. Biol. Med.* 36, 1434–1443. doi: 10.1016/j.freeradbiomed.2004.03.010

Weyer, K., Storm, T., Shan, J., Vainio, S., Kozyraki, R., Verroust, P. J., et al. (2011). Mouse model of proximal tubule endocytic dysfunction. *Nephrol. Dial Transplant.* 26, 3446–3451. doi: 10.1093/ndt/gfr525

Windhager, E. E. (1992). *Section 8: Renal Physiology*. Oxford: Oxford University Press.

Wirthensohn, G., and Guder, W. G. (1986). Renal substrate metabolism. *Physiol. Rev.* 66, 469–497. doi: 10.1152/physrev.1986.66.2.469

Wish, J. B., Aronoff, G. R., Bacon, B. R., Brugnara, C., Eckardt, K. U., Ganz, T., et al. (2018). Positive iron balance in chronic kidney disease: How much is too much and how to tell? *Am. J. Nephrol.* 47, 72–83. doi: 10.1159/000486968

Wittig, I., and Schagger, H. (2008). Structural organization of mitochondrial ATP synthase. *Biochim. Biophys. Acta* 1777, 592–598. doi: 10.1016/j.bbabio.2008.04.027

Wolff, N. A., Garrick, L. M., Zhao, L., Garrick, M. D., and Thévenod, F. (2014a). Mitochondria represent another locale for the divalent metal transporter 1 (DMT1). *Channels* 8, 458–466. doi: 10.4161/19336950.2014.956564

Wolff, N. A., Garrick, M. D., Zhao, L., Garrick, L. M., Ghio, A. J., and Thévenod, F. (2018). A role for divalent metal transporter (DMT1) in mitochondrial uptake of iron and manganese. *Sci. Rep.* 8:211.

Wolff, N. A., Ghio, A. J., Garrick, L. M., Garrick, M. D., Zhao, L., Fenton, R. A., et al. (2014b). Evidence for mitochondrial localization of divalent metal transporter 1 (DMT1). *FASEB J.* 28, 2134–2145. doi: 10.1096/fj.13-240564

Wolff, N. A., Liu, W., Fenton, R. A., Lee, W. K., Thévenod, F., and Smith, C. P. (2011). Ferroportin 1 is expressed basolaterally in rat kidney proximal tubule cells and iron excess increases its membrane trafficking. *J. Cell. Mol. Med.* 15, 209–219. doi: 10.1111/j.1582-4934.2009.00985.x

Wu, X., Su, S., Zhai, R., Chen, K., Jin, T., Huang, B., et al. (2004). Lack of reversal effect of EDTA treatment on cadmium induced renal dysfunction: a fourteen-year follow-up. *Biometals* 17, 435–441. doi: 10.1023/b:biom.0000029440.23884.d6

Yan, J., Jiang, J., He, L., and Chen, L. (2020). Mitochondrial superoxide/hydrogen peroxide: an emerging therapeutic target for metabolic diseases. *Free Radic. Biol. Med.* 152, 33–42. doi: 10.1016/j.freeradbiomed.2020.02.029

Yanatori, I., and Kishi, F. (2019). DMT1 and iron transport. *Free Radic. Biol. Med.* 133, 55–63. doi: 10.1016/j.freeradbiomed.2018.07.020

Yanatori, I., Yasui, Y., Tabuchi, M., and Kishi, F. (2014). Chaperone protein involved in transmembrane transport of iron. *Biochem. J.* 462, 25–37. doi: 10.1042/bj20140225

Youle, R. J., and Van Der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science* 337, 1062–1065. doi: 10.1126/science.1219855

Yu, H. T., Zhen, J., Xu, J. X., Cai, L., Leng, J. Y., Ji, H. L., et al. (2020). Zinc protects against cadmium-induced toxicity in neonatal murine engineered cardiac tissues via metallothionein-dependent and independent mechanisms. *Acta Pharmacol. Sin.* 41, 638–649. doi: 10.1038/s41401-019-0320-y

Zainal, T. A., Weindruch, R., Szweda, L. I., and Oberley, T. D. (1999). Localization of 4-hydroxy-2-nonenal-modified proteins in kidney following iron overload. *Free Radic. Biol. Med.* 26, 1181–1193. doi: 10.1016/s0891-5849(98)00312-8

Zelko, I. N., Mariani, T. J., and Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* 33, 337–349. doi: 10.1016/s0891-5849(02)00905-x

Zotova, L., Aleschko, M., Sponder, G., Baumgartner, R., Reipert, S., Prinz, M., et al. (2010). Novel components of an active mitochondrial K(+)/H(+) exchange. *J. Biol. Chem.* 285, 14399–14414. doi: 10.1074/jbc.m109.059956

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 03 September 2020
doi: 10.3389/fphys.2020.01050

Dyslipidemia in Kidney Disorders: Perspectives on Mitochondria Homeostasis and Therapeutic Opportunities

Pei-Hui Lin[1]* *and Pu Duann*[2]

[1]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, United States, [2]Research and Development, Salem Veteran Affairs Medical Center, Salem, VA, United States

To excrete body nitrogen waste and regulate electrolyte and fluid balance, the kidney has developed into an energy factory with only second to the heart in mitochondrial content in the body to meet the high-energy demand and regulate homeostasis. Energy supply from the renal mitochondria majorly depends on lipid metabolism, with programed enzyme systems in fatty acid β-oxidation and Krebs cycle. Renal mitochondria integrate several metabolic pathways, including AMPK/PGC-1α, PPARs, and CD36 signaling to maintain energy homeostasis for dynamic and static requirements. The pathobiology of several kidney disorders, including diabetic nephropathy, acute and chronic kidney injuries, has been primarily linked to impaired mitochondrial bioenergetics. Such homeostatic disruption in turn stimulates a pathological adaptation, with mitochondrial enzyme system reprograming possibly leading to dyslipidemia. However, this alteration, while rescuing oncotic pressure deficit secondary to albuminuria and dissipating edematous disorder, also imposes an ominous lipotoxic consequence. Reprograming of lipid metabolism in kidney injury is essential to preserve the integrity of kidney mitochondria, thereby preventing massive collateral damage including excessive autophagy and chronic inflammation. Here, we review dyslipidemia in kidney disorders and the most recent advances on targeting mitochondrial energy metabolism as a therapeutic strategy to restrict renal lipotoxicity, achieve salutary anti-edematous effects, and restore mitochondrial homeostasis.

Keywords: fatty acid β-oxidation, energy metabolism, lipotoxicity, podocyte, proximal tubule cells, oxidative stress, homeostasis, fibrosis

OPEN ACCESS

Edited by:
Giuliano Ciarimboli, University of Münster, Germany

Reviewed by:
Michele Visentin, University Hospital Zürich, Switzerland
Melinda Coughlan, Monash University, Australia

***Correspondence:**
Pei-Hui Lin
pei-hui.lin@osumc.edu; plin1999@yahoo.com

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *17 May 2020*
Accepted: *30 July 2020*
Published: *03 September 2020*

Citation:
Lin P-H and Duann P (2020) Dyslipidemia in Kidney Disorders: Perspectives on Mitochondria Homeostasis and Therapeutic Opportunities. Front. Physiol. 11:1050. doi: 10.3389/fphys.2020.01050

INTRODUCTION

The kidney is characterized by a complex anatomy, with millions of nephrons as the functional unit to excrete nitrogen waste and secure fluid homeostasis. The kidney is composed of multiple specialized cell types ensuring vital homeostasis of acid-base and electrolyte balance, blood pressure regulation, nutrient reabsorption, and hormone secretion (Hoenig and Zeidel, 2014; Duann and Lin, 2017; Yu et al., 2019). Therefore, it is one of the most metabolically active organs other than heart and skeletal muscle, with proximal tubules presenting a very high density of mitochondria required for energy consumption (Meyer et al., 1997; Wang et al., 2000).

For example, the human proximal convoluted tubules (S1 and S2 combined) contain abundant large mitochondria, which occupy about 16.3% cell volume (Møller and Skriver, 1985). Notably, the mature nephron comprises distinct segments, each utilizing metabolic pathways to varying degrees depending on the specific function (Cargill and Sims-Lucas, 2020).

Complete oxidation of fatty acids (FAs), which are high-energy substrates, to CO_2 and H_2O gives rise to roughly 9 Kcal/g fat, while only 4 Kcal/g are generated from carbohydrates or proteins. The heart possesses metabolic flexibility and powerful catabolic capacity to use various energy substrates, mainly FAs (40–60%) and glucose (20–40%), for ATP production (Karwi et al., 2018). Instead, most proximal tubule epithelial cells (PTEC) have low metabolic flexibility toward glycolysis and rely on FAs as energy source at baseline (Bonventre and Yang, 2011). This was shown by early *in vivo* studies measuring ATP synthesis by tracking isotope-labeled FAs with NMR in rat kidney, which indicated that FAs are a preferred fuel (Freeman et al., 1986). However, PTEC are able to shift to anaerobic glycolysis to produce ATP required for cellular regeneration after ischemic acute kidney injury (AKI; Lan et al., 2016). In mice, glomerular podocytes display much lower mitochondrial density than in PTECs and rely primarily on anaerobic glycolysis to maintain glomerular filtration barrier and are relatively insensitive to defect in mitochondrial biogenesis during ischemia damage (Brinkkoetter et al., 2019). Instead, as lipid accumulation is commonly observed in patients with chronic kidney disease, podocytes are rather sensitive to cellular cholesterol-mediated glomerular injury (Merscher et al., 2014).

Mitochondria are pivotal for maintaining the health and function of the metabolically active kidney by providing efficient energy support through the process of oxidative phosphorylation (OXPHOS) and aerobic glycolysis. Several factors such as mitochondria biogenesis, bioenergetics, dynamics, and autophagy regulate the mitochondrial physiology (Duann and Lin, 2017). In addition, mitochondria also contribute to production of reactive oxygen species (ROS) free radicals and transduction of metabolic and stress signals (Galvan et al., 2017; Flemming et al., 2018). Persistent mitochondrial damage is a major source of oxidants. Consequently, mitochondrial fitness translates into body's general health. Mitochondrial dysfunction is involved in various kidney diseases, such as acute kidney injury (AKI), chronic kidney disease (CKD), diabetic nephropathy (DN), and glomerulonephritis (GN; Duann and Lin, 2017; Eirin et al., 2017; Galvan et al., 2017; Flemming et al., 2018).

Dysregulated lipid metabolism with defective cholesterol/free fatty acid (FFA) metabolism leading to dyslipidemia is common in patients of several kidney diseases, including acute kidney disease, CKD, diabetic kidney disease (DKD), nephrotic syndrome, and uremia (Agrawal et al., 2017; Hager et al., 2017; Gai et al., 2019; Nishi et al., 2019; Nishi and Nangaku, 2019; Jang et al., 2020b), and may contribute to end-stage kidney disease. In this review, we summarize the recent advances in understanding lipid metabolism in the function of kidney mitochondria and the molecular mechanisms related to dyslipidemia during kidney disease progression.

BASIC LIPID BIOLOGY

In biological systems, lipids include fats, sterols, phospholipids, and triacylglycerides (TAG). In the cell, lipids have numerous functions: they constitute the cell membrane as a protective barrier; form membranous compartments of intracellular organelles; provide energy source and storage; provide building blocks for hormones; and serve as secondary cellular messengers within body. FAs are carboxylic acids with a long aliphatic tail, which constitute building blocks for other lipids such as TAG and phospholipids.

Within the body, lipid metabolism comprises several inter-dependent pathways for the generation, storage, and transport of lipids, which involves plasma lipoprotein particles [chylomicrons, high density lipoproteins (HDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), and very low density lipoproteins (VLDL)] in circulation. Dietary lipids, mainly (95%) TAG, some FFAs, and cholesterol, carried by chylomicrons into circulation, are degraded into FFAs and glycerol by lipoprotein lipase (LPL) activity on the capillaries. These FFAs are taken up by muscle, heart, and adipose and peripheral tissues like kidney; remnants of chylomicrons are subsequently cleared in the liver (Florens et al., 2016; Agrawal et al., 2017; Kronenberg, 2018). FFAs are transported by serum albumin to the liver and periphery and could be stored as TAG in kidney capillaries. Additionally, esterified cholesterol could be stored as a lipid droplet within the kidney.

TRANSPORT OF CELLULAR FFAs

Lipid Uptake by CD36 in the Kidney

FA uptake from the extracellular milieu is the first step in their utilization. Multiple cell surface lipid transport proteins, such as cluster of differentiation 36 (CD36), scavenger receptor B1 (SR-B1), tissue-specific fatty acid transport proteins (FATPs), and plasma membrane fatty acid-binding protein ($FABP_{pm}$) facilitate cellular FFA uptake (Haunerland and Spener, 2004; Su and Abumrad, 2009; **Figure 1**).

Long-chain fatty acids (LCFAs, referring to FAs with 12 or longer carbons chains) primarily enter the cell *via* FA transporter CD36 [also known as Fatty acid translocase (FAT) or SR-B2]. CD36 is expressed in multiple cell types and mediates diverse functions, such as lipid uptake, inflammation, ROS production, molecular adhesion, and apoptosis. CD36 is a multifunctional receptor for many ligands, including collagen, native lipoproteins, LCFA, oxidized phospholipids, oxidized LDL, thrombospondin, and apoptotic cells (Yang et al., 2017; Wang and Li, 2019). Several post-translational modifications, including phosphorylation, palmitoylation, ubiquitylation, and glycosylation regulate CD36 stability and dimerization, and correlate its function to myocellular FA uptake (Luiken et al., 2016). In adipocytes, two palmitoyl-acyltransferases (PATs), namely DHHC4/5, modulate CD36 palmitoylation and target it to the plasma membrane lipid rafts, where it mediates FA adsorption and transport (Wang et al., 2019). Interestingly, in addition to the cell surface, CD36 also localizes to the ER, endosomes, and mitochondria (Bonen et al., 2000; Smith et al., 2011). In response to diverse signaling transduction

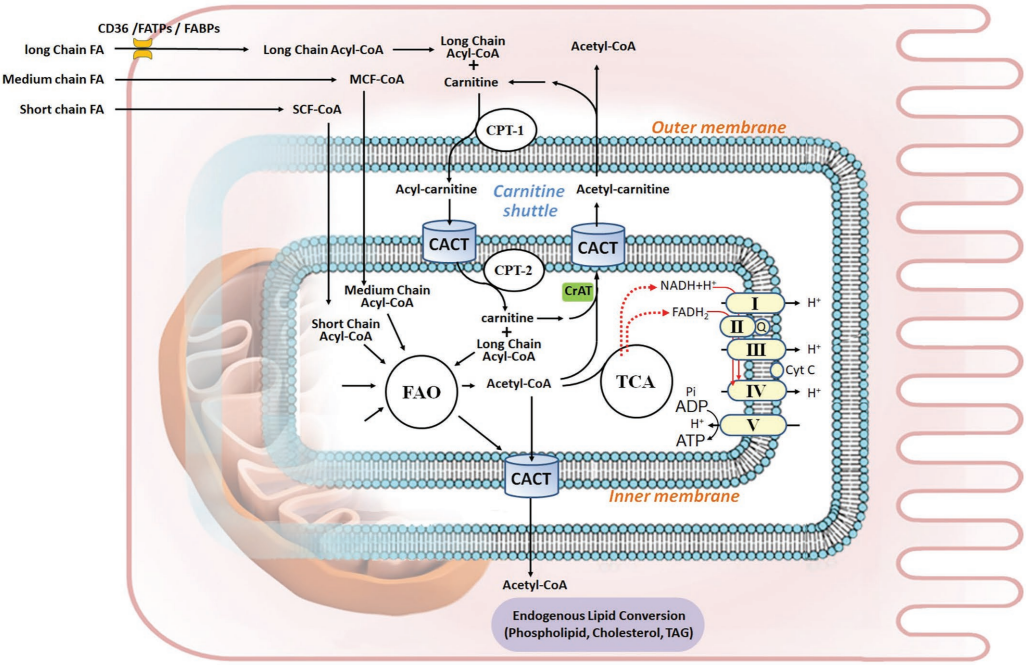


FIGURE 1 | Fatty acid metabolism in renal proximal tubule epithelial cell. Fatty acids (FAs) are the preferred energy substrates for the kidney. Uptake of FAs from capillaries into kidney cells is facilitated by either FAT/CD36 or FABPs and FATPs. In the cytosol, FAs are activated to acyl-CoA, esterified with carnitine, and transported into the mitochondrial matrix through the carnitine shuttle, which is composed of CPT-1, CACT, and CPT-2. Medium-chain FAs and short-chain FAs can permeate the mitochondrial membranes. In the matrix, acyl-CoA undergoes FA β-oxidation (FAO), thereby generating acetyl-CoA to fuel the TCA cycle, as well as $FADH_2$ and NADH that serve as electron donors to the five ETC complex for ATP production *via* oxidative phosphorylation. Acetyl-CoA can be shuttled out of mitochondria through carnitine acetyltransferase (CrAT), while it goes through integrated endogenous lipid conversion to form phospholipids, cholesterol, and triacylglycerol (TAG). Q, Coenzyme Q; Cyt C, cytochrome C.

pathways, rapid mobilization of the vesicular transport system mediates dynamic intracellular distribution of CD36 to reprogram energy utilization and control lipid metabolism (Georgiou et al., 2015; Glatz et al., 2016).

In the kidney, CD36 mediates FA uptake and lipid metabolic reprograming and functions (Yang et al., 2017). CD36 is highly expressed in mesangial cells (Ruan et al., 1999), renal proximal (Susztak et al., 2005) and distal tubular epithelial cells (Okamura et al., 2007), podocytes (Hua et al., 2015), microvascular endothelial cells, and interstitial macrophages (Rahman et al., 2008; Kennedy et al., 2013). Transgenic mice with tubular overexpression of CD36 demonstrate tubular-specific accumulation of lipids, TAG, and LCFAs (Kang et al., 2015). Other kidney CD36 substrates include oxidized phospholipids, advanced oxidation protein products (AOPPs; Li et al., 2019), and advanced glycation end products (AGEs), which promote inflammation, ER stress, and renal cells apoptosis and contribute to renal fibrosis (Okamura et al., 2009; Ruggiero et al., 2014; Pennathur et al., 2015).

Lipid Uptake by Other Transporters in the Kidney: FABPs and FATPs

FA-binding proteins (FABPs) are low molecular weight (14–15 kDa) proteins that transport LCFAs through cell membranes, transport FAs to mitochondria and peroxisomes, and function as chaperones to mediate intracellular transport. Two major FABP isoforms are expressed in human kidneys, the proximal tubule-enriched FABP1 (also known as liver type L-FABP) and the distal tubule-enriched FABP3 (Maatman et al., 1991). Urinary FABP1 level was proposed as a biomarker of acute tubulointerstitial damage (Yamamoto et al., 2007; Pelsers, 2008).

Emerging data also support proximal tubular apical expression of FA transporter-2 (FATP2, encoded by *Slc27a2*) and its role in luminal non-esterified FA (NEFA) reabsorption from glomerular filtrate and NEFA metabolism in mice. Silencing of FATP2 in human renal PTEC *in vitro* leads to increased Oil Red O staining and subsequent apoptosis following FA exposure. Moreover, tubular lipoapoptosis in lipidated albumin-injected mice decreases in *Slc27a2*-deficient mice. These data suggest that luminal NEFA uptake by FATP2 causes proximal tubule lipoapoptosis, which may contribute to tubular atrophy and CKD progression (Khan et al., 2018).

FATTY ACID METABOLISM IN KIDNEY MITOCHONDRIA

FA β-oxidation (FAO) may occur in both mitochondria and peroxisomes. While mitochondria majorly oxidize LCFAs, and medium-chain and short-chain FAs (MCFAs and SCFAs, referring to FAs with less than 12 carbons chains), peroxisomes oxidize specific carboxylic acids such as very long-chain FAs (VLCFAs), branched-chain FAs, fatty dicarboxylic acids, and

bile acid intermediates (in the liver; Cipolla and Lodhi, 2017). Interestingly, peroxisomal FAO provides alternative metabolism of LCFAs and MCFAs in case of mitochondrial long-chain FAO deficiencies (Violante et al., 2019). Mitochondrial FAO is thus the major pathway for the degradation of FAs to sustain cellular energy homeostasis (Houten et al., 2016). This process includes six tightly-regulated steps: (i) FA esterification to acyl-CoA; (ii) mitochondrial CPT shuttle (or the carnitine shuttle); (iii) the FAO pathway; (iv) the OXPHOS pathway; (v) allosteric control of FAO; and (vi) integrated nutrient metabolism in the kidney (**Figure 1**).

Fatty Acid Esterification to acyl-CoA

FAs must be converted to fatty acyl-CoA by cytosolic acyl-CoA synthetases in order to enter mitochondria. Once inside the cell, MCFAs or SCFAs can freely diffuse into mitochondria. However, LCFAs need to be activated to long-chain acyl-CoA (LC acyl-CoA) and esterified with carnitine into LC-acylcarnitine to permeate the outer mitochondrial membrane (OMM) and subsequently be transported into the mitochondrial matrix (Bremer, 1983).

The Carnitine Shuttle

The carnitine shuttle, mediated by the rate-limiting enzyme carnitine palmitoyltransferase I (CPT-1, on the OMM) and the two inner mitochondrial membrane (IMM) proteins carnitine-acylcarnitine translocase (CACT) and carnitine palmitoyltransferase II (CPT-2), serves to transport the FA moiety into mitochondria. CPT-2 conducts a reverse reaction to convert LC-acylcarnitine back to LC acyl-CoA and carnitine. Carnitine is transported back to the cytoplasm by the same shuttle (Brivet et al., 1999).

The FAO Pathway

FAO is the process of breaking down a LC acyl-CoA into acetyl-CoA molecules inside the mitochondrial matrix. The term β-oxidation refers to the position of the carbon group being oxidized. The number of acetyl-CoA molecules produced depends on the initial carbon length of the FA. When LC acyl-CoA enters FAO, two carbons are cleaved to generate an acetyl-CoA and an acyl-CoA that is two carbons shorter from each β-oxidation cycle. This process continues until all of the carbons in the FA are turned into acetyl-CoA to fuel the tricarboxylic acid (TCA) cycle and generate ATP. The two redox active coenzymes – the reduced form of nicotinamide adenine dinucleotide (NADH) and the hydroquinone form of flavin adenine dinucleotide ($FADH_2$) – produced during each β-oxidation cycle, along with those generated from TCA cycle, are used as electron donors by the electron transport chain (ETC) complex, in the redox reaction that produces ATP (the OXPHOS pathway). LCFA oxidation yields high energy: for instance, 137 ATP are generated from palmitate as opposed to 38 obtained from glucose oxidation (Nsiah-Sefaa and McKenzie, 2016).

The OXPHOS Pathway

The mitochondrial ETC/OXPHOS respiratory chain contains five complexes. Complexes I–IV transfer electrons (e^-) and protons (H^+) across IMM to generate an electrochemical gradient for ATP synthesis in complex V (ATP synthase). Several critical steps regulate this process. The concentration of NAD constitutes the rate-limiting process (Canto et al., 2015; Verdin, 2015). Coenzyme Q10 (CoQ10) is a component of ETC, which shuttles electrons in the respiratory chain. Moreover, the reduced form of CoQ10 is also a potent antioxidant (Ernster and Dallner, 1995; Thomas et al., 1996). CoQ deficiency could cause nephropathies (Ozaltin, 2014) and mutation in ADCK4 (CoQ8B), a protein required for stabilizing CoQ complex in podocyte, is an etiology of steroid-resistant nephrotic syndrome (SRNS or FSGS; Ashraf et al., 2013; Widmeier et al., 2020). Cardiolipin, an IMM phospholipid, plays a central structural role in cristae formation, facilitates ETC supra-complex formation for optimal OXPHOS activity, and serves as a platform to initiate apoptosis (Birk et al., 2014; O'Brien et al., 2015).

Allosteric Control of FAO

Mitochondrial bioenergetic homeostasis is subjected to allosteric regulation by the ratios of the [Acetyl CoA/CoA], [NADH/NAD^+], and [$FADH_2$/FAD^+]. Therefore, FAO enzymatic activities are affected by the levels of the metabolic products of their own reactions, and a rise in [Acetyl-CoA/CoA] or [NADH/NAD^+] leads to feedback inhibition of FAO (Karwi et al., 2019). For example, mice with proximal tubule-specific deletion of carnitine acetyltransferase (CrAT), an enzyme that controls inter-conversion of Acetyl-CoA/CoA and shuttles excess FA products out of the mitochondria, develop mitochondrial dysfunction, cellular apoptosis, and tubular and glomerular fibrosis (Kruger et al., 2019). Interestingly, *de novo* synthesis of NAD^+, a central metabolic coenzyme/co-substrate involved in cellular energy metabolism, profoundly affects mitochondrial fitness in organ health and injury, including kidney (Hershberger et al., 2017; Katsyuba et al., 2018; Poyan Mehr et al., 2018; Ralto et al., 2020).

Integrated Nutrient Metabolism in the Kidney

Acetyl-CoA is a critical metabolite derived from catabolism of all major nutrient sources, such as glucose, FAs, and amino acids. Moreover, acetyl-CoA can be diverted from the TCA cycle to synthesize cholesterol, phospholipids, and TAG in the cell (Pietrocola et al., 2015; Shi and Tu, 2015). Proper integration and regulation of energy metabolism during ATP loss or excess are thus key to maintain mitochondrial health during injury and repair in renal pathophysiology (Vamecq et al., 2012; Aon et al., 2014; Fornoni et al., 2014; Szeto, 2017; Jang et al., 2020a).

TRANSCRIPTIONAL, EPIGENETIC, AND POST-TRANSLATIONAL REGULATION OF FAO AND MITOCHONDRIA BIOGENESIS

Several transcriptional, epigenetic, and post-translational regulators are involved in the crosstalk between peroxisomes, nucleus, and mitochondria to control the expression/functions of FAO enzymes, mitochondrial biogenesis, and energy reprograming in health and disease-stressed states (Stallons et al., 2013; Bhargava and Schnellmann, 2017; **Figure 2**).

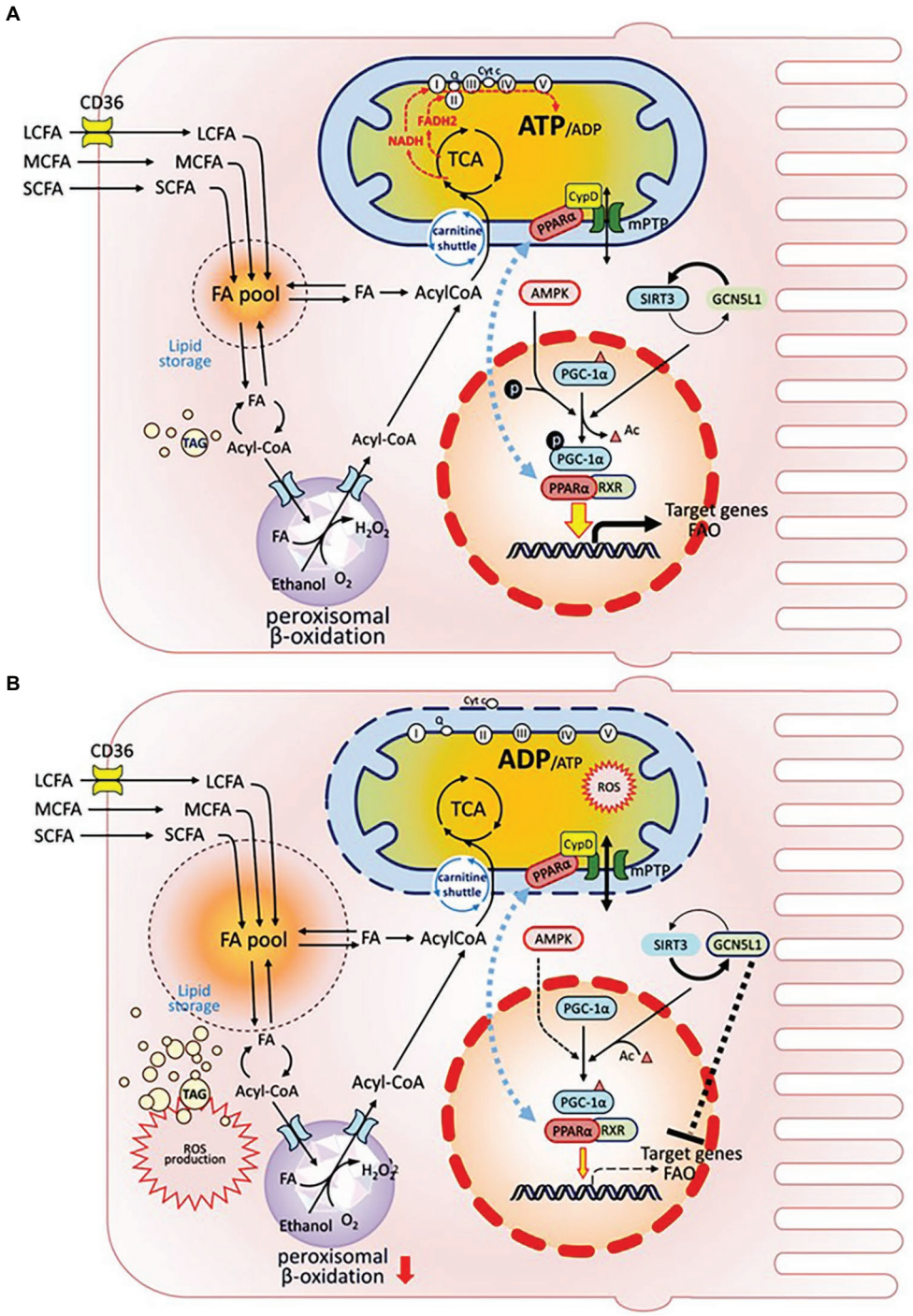


FIGURE 2 | Organelle crosstalk regulates fatty acids metabolism in renal PTEC under healthy or injury/disease states. Intracellular FA metabolism includes catabolic and anabolic pathways. FAs are oxidized either in mitochondria or peroxisome to generate ATP (catabolism), or are stored as global triglyceride pool (anabolism). PGC-1α is the mitochondrial master regulator, which drives mitochondrial biogenesis by co-activating transcriptional factors PPAR-α and RXR to regulate the expression of target genes affecting biogenesis, OXPHOS, and FAO. PGC-1α is also extensively regulated by post-translational modifications: PGC-1α is activated *via* phosphorylation by AMPK; its acetylation state is regulated by the counter-balance between SIRT deacetylase and GCN5L1 acetylase. GCN5L1 activation also negatively modulates FAO target genes. Translocation of PPARα between nucleus and IMM affects PPARα activity as a transcription factor. **(A)** In healthy condition. **(B)** Under injury/disease state, impaired PGC-1α leads to defective FAO and is associated with reduced FA catabolism, increased FA pool and TAG accumulation, increased cellular ROS production, and PPARα mitochondrial translocation, which induces PPARα interaction with CypD, mPTP opening, ETC disruption, cytochrome C release, and mitochondrial damage.

These key molecules include the nuclear hormone receptor, peroxisome proliferator-activated receptors (PPARs, PPARα, and PPARγ as examples; Wu et al., 2009; Corrales et al., 2018); PPARγ coactivator 1α (PGC-1α; Weinberg, 2011; Li and Susztak, 2018; Fontecha-Barriuso et al., 2020); the NAD^+-dependent deacetylases sirtuins (SIRTs; Wakino et al., 2015; Hershberger et al., 2017; Morigi et al., 2018); AMP-activated protein kinase (AMPK); and nuclear respiratory factors 1 and 2 (NRF1 and NRF2; Akhtar and Siragy, 2019).

PGC-1α, the mitochondrial biogenesis master regulator, is predominantly expressed in proximal tubules and interacts directly with multiple transcription factors to integrate upstream signaling events with mitochondrial biogenesis and functional capacity. Downstream transcription factors control all aspects of mitochondrial function, including biogenesis, energy production, dynamics, and protein homeostasis. PGC-1α regulates the expression of NRF1 and NRF2 to increase NAD^+ biosynthesis (Tran et al., 2016) and activates genes coding for the OXPHOS system (Zoja et al., 2014). In obesity-related nephropathy models, reduced NRF2 along with suppressed expression of the key FAO enzyme long-chain acyl-CoA synthetase-1 (ACSL1) are associated with elevated renal lipid deposition, further supporting the importance of mitochondria in lipid metabolism and energy homeostasis (Chen et al., 2019).

As the PGC-1α/PPARα axis governs transcriptional regulation of FAO, it was proposed as therapeutic target in AKI and CKD (Simon and Hertig, 2015; Stadler et al., 2015). Defects in the FAO pathway, such as reduced expression of CPT-1 (Kang et al., 2015), CrAT (Kruger et al., 2019), and PPARα (Chung et al., 2018) are associated with CKD and renal fibrosis. PPARα heterodimerizes with its obligate partner, the retinoid-X-receptor (RXR), to regulate FAO and energy metabolism. FAs are natural activators of PPARα, and one of PPARα target genes is CD36 (which increases FA uptake; Portilla, 2003). Moreover, impaired expression of PPARα and specific proteins in FAO pathway are associated with lipid accumulation and fibrosis in renal tubular epithelial cells in aging rats (Chung et al., 2018). Interestingly, Jang et al. demonstrated that proximal tubular mitochondrial interaction of PPARα with cyclophilin D (CypD), a component of the IMM structural protein complex mitochondrial permeability transition pore (mPTP), could repress nuclear PPARα activity and negatively modulate FAO in cisplatin-induced AKI (Jang et al., 2020a). Several PPARα agonists have been shown to enhance FAO activity in kidney (Konig et al., 2008; Lakhia et al., 2018).

PGC-1α is extensively regulated by post-translational modifications. AMPK and SIRT positively regulate PGC-1α through phosphorylation or deacetylation, respectively (Jager et al., 2007; Canto and Auwerx, 2009). Interestingly, liver histone demethylase JMJD3 was identified as a gene-specific transcriptional partner of SIRT1 that epigenetically activates mitochondrial β-oxidation during fasting (Seok et al., 2018). The counterpart of JMJD3 in kidney remains to be uncovered. General control of amino acid synthesis-5-like 1 (GCN5L1), a protein acetylase counteracting the function of SIRT3, was recently shown to negatively modulate hepatic FAO enzyme activities *via* acetylation (Thapa et al., 2018). Similarly, GCN5L1-mediated hyper-acetylation and impairment of FAO enzymes might be a key pathogenic event underlying lipid overload-induced kidney injury (Lv et al., 2019).

In summary, the integrated regulation of FA metabolism at the genetic, epigenetic, and protein level is tightly associated with mitochondrial homeostasis. Under injury or disease state, deficiency in PGC-1α and associated transcription factors leads to defective FAO, enlarged FA pool and TAG accumulation, massive ROS production, increased PPARα mitochondrial translocation inducing mPTP opening and loss of mitochondrial membrane potential, cytochrome C release, and mitochondrial damage (**Figure 2**).

DYSLIPIDEMIA AND CELLULAR LIPOTOXICITY-MEDIATED KIDNEY INJURY

Dyslipidemia is an abnormal amount of lipids (e.g., TAG, cholesterol or phospholipids) in the blood. Lipids in excess, which is delivered to organs beyond their energy demands, can be stored mainly as TAG in intracellular lipid droplets (LDs), an ubiquitous organelle that serves as energy stores, dynamic membrane synthesis, and as a hub for further metabolic regulation (Walther et al., 2017). Accumulation of such lipid intermediates or final products in non-adipose tissues, along with the subsequent multi-factorial disturbance of intracellular homeostasis, could result in lipotoxicity of target tissues. Lipotoxicity thus represents a pathologic phenomenon with hallmarks of aberrant lipid accumulation, causing metabolic, inflammatory, oxidative stress in intracellular organelles, and further triggering cell damages (Su et al., 2017; Opazo-Ríos et al., 2020).

Moorhead et al. first hypothesized "lipid nephrotoxicity" in 1982, proposing that dyslipidemia may contribute to the progression of renal dysfunction (Moorhead et al., 1982). This hypothesis had gained supportive evidence in several contexts. For examples, renal lipid accumulation has been shown with high clinical prevalence in patients with CKD, including the insulin resistant obese subjects with diabetic nephropathy (Herman-Edelstein et al., 2014; Escasany et al., 2019; Opazo-Ríos et al., 2020), in nephrotic syndrome (Vaziri, 2016; Agrawal et al., 2017), focal segmental glomerulosclerosis (FSGS; Sasaki et al., 2018), and also as a consequence of acute ischemic renal injury (Zager et al., 2011). Significant alterations in renal lipid metabolism are typified as high TAG, variation in the composition of apolipoproteins and lipids, the accumulation of atherogenic particles VLDL and IDL, and decreased HDL cholesterol (Vaziri, 2006; Stadler et al., 2015; Florens et al., 2016; Kronenberg, 2018; Du and Ruan, 2019; Gai et al., 2019; Thongnak et al., 2020; Jang et al., 2020b). As discussed earlier, systematic lipid metabolism involves multi-organ crosstalk, ultimately also affecting kidney function. Therefore, dyslipidemia and lipid nephrotoxicity could be not only a consequence but also a cause of kidney disease (Florens et al., 2016; Agrawal et al., 2017; Kronenberg, 2018; Czumaj et al., 2019; Nishi et al., 2019).

Excess fat could be derived from either dysfunctional capacity of adipose lipid storage, or from diet-induced hyperlipidemia (high plasma albumin-bound FFAs and cholesterol), or in the condition of renal dysfunction (as commonly exemplified by renal mass reduction in animal model) and defective insulin signaling. Excess kidney ectopic fat deposition and lipid overload in intracellular organelles could lead to ER stress (Zhao et al., 2008), mitochondria dysfunction (Vamecq et al., 2012; Szeto et al., 2016), and lysosomal stress (Yamamoto et al., 2017, 2020). These alterations could change cellular protective mechanisms such as autophagy, mitophagy, lipophagy and contribute to apoptosis and cell damage. These observations thus support the notion of dyslipidemia contributes to the progression of renal injury, and lipid-lowering therapies or shielding mitochondria could provide beneficial effects on lipotoxicity-mediated kidney injury (Izquierdo-Lahuerta et al., 2016; Su et al., 2017).

Dyslipidemia could appear in various forms with different causes and consequences. In lipid-mediated podocyte damage, FFAs and their metabolism affect function and survival of podocytes (Sieber and Jehle, 2014). Dyslipidemia is also a common feature, rather than a complication, of nephrotic syndrome. Excessive urinal protein loss results in hypoproteinemia, in turn leading to low serum oncotic pressure, and even edematous change in severe cases. To rescue the oncotic pressure deficit, the body initiates a reactive hepatic protein synthesis, including lipoproteins (Attman and Alaupovic, 1990; Merscher et al., 2014; Vaziri, 2016; Agrawal et al., 2017). Additionally, reduced plasma levels of lipoprotein lipase results in decreased lipid catabolism. Elevated serum levels of LDL and IDL are filtered through glomeruli and lead to lipiduria, which manifests with fatty casts containing oval fat bodies in the urine sediment (Cavanaugh and Perazella, 2019).

Mutations affecting cholesterol metabolism in the process of lipid trafficking, storage, influx, or efflux, could mediate glomerular injury (Merscher et al., 2014). For example, Tangier disease (OMIM #205400) or DKD caused by mutations in ATP-binding cassette A1 (ABCA1) gene result in reduced HDL in circulation, albuminuria, podocyte phenotype with esterified cholesterol accumulation and dysfunctional mitochondria due to cardiolipin hyperoxidation (Ducasa et al., 2019a,b). For the topics on glomerular diseases-related renal lipotoxicity and mitochondrial dysfunction, please refer to the comprehensive review in the same special issue (Ge et al., 2020).

Furthermore, excess of FFAs leads to TAG accumulation and renal tubular toxicity (Johnson et al., 2005; Scerbo et al., 2017). Increased LCFA-bound albumin induces altered redox balance, high tubular cell apoptosis, and kidney fibrosis (Ruggiero et al., 2014). As lipoprotein abnormalities also correlate with high risk of both cardiovascular and kidney diseases, these modified lipoproteins could be accounted as actual mediators of uremic toxicity (Florens et al., 2016). LDL and oxidized (ox)-LDL uptake by mesangial cells lead to cell proliferation and glomerular matrix expansion, while uptake by PTE results in tubulointerstitial lesions with remarks of heightened expression of extracellular matrix proteins (Nosadini and Tonolo, 2011). HDL is a key player in reverse cholesterol transport to shuttle cholesterol from peripheral cells, such as macrophages, to the liver, therefore relieving the cholesterol burden of these cells. HDL thus exerts its anti-oxidant function through preventing LDL oxidation by ROS and protecting against the adverse effects of ox-LDL on the endothelium. Reduced levels and dysfunction of HDL, which could be due to perturbed HDL proteome composition, are common in CKD patients (Vaziri, 2006; Yamamoto et al., 2012; Agrawal et al., 2017; Kronenberg, 2018; Rysz et al., 2020).

Deficiency of FA metabolism and lipid overload are the main drivers in the progression of both glomerular and tubular kidney diseases. Lipid accumulation, particularly in ischemic proximal tubules, may result in persistent energy depletion with FFA-induced mitochondrial dysfunction, which could play an important role in the AKI to CKD transition (Szeto, 2017). Conversely, mitochondrial protection prevents high-fat diet-induced glomerular and tubular lesions (Szeto et al., 2016).

The pathophysiological changes underlying hyperlipidemia may involve energy shortage from impaired mitochondrial biogenesis or ATP energetics, and systemic oxidative stress due to excessive ROS production accompanied by ER stress and influx of inflammatory cytokines. Without timely intervention, these changes could eventually lead to apoptosis and kidney fibrosis (Agrawal et al., 2017; Du and Ruan, 2019). Less is known about the molecular mechanism of some toxic lipid intermediates ("metabolic poison") derived from deficiency or decreased expression of FAO-related enzymes in kidney disease development (Stadler et al., 2015; Su et al., 2017); and future research may elucidate this process.

TARGETING MITOCHONDRIAL ENERGY METABOLISM AND LIPOTOXICITY IN KIDNEY DISEASES

Lipid-lowering therapies in kidney diseases have been studied for many years, although statins is still the first choice of conventional hypolipidemia strategies for its effect on HMGCoA inhibition to block cholesterol synthesis. Cumulative pharmacological efforts have advanced the field to develop classic and novel lipid modifying therapies in kidney diseases, as extensively reviewed recently (Ferro et al., 2018; Sudhakaran et al., 2018; Filippatos et al., 2019; Rosenson et al., 2019; Heine et al., 2020; Opazo-Ríos et al., 2020). These include effective and well-tolerated drugs targeting various lipid synthesis, uptake, trafficking and metabolism pathways. Recent years, compounds that specifically target mitochondria have emerged as promising therapeutic options for patients with renal disease. Here, we discuss molecules targeting mitochondrial lipid metabolism and mitochondrial dysfunction pathways, including pharmacological agents promoting mitochondrial FAO, mitochondrial biogenesis, and ATP synthesis, as well as mitochondrial antioxidants (regulating ROS metabolism) and cardiolipin stabilizers.

Mitochondrial FAO-Promoting Agents

Carnitine and acetyl-L-carnitine are nonessential nutrients, as kidney of healthy subjects normally produce sufficient carnitine from daily food intake/metabolism and preserve its excretion well. However, carnitine could be used as dietary supplements to help with carnitine shuttle of FAO in conditions of "primary carnitine deficiency" (children with genetic disorder of carnitine transporter OCTN2 encoded by the *SLC22A5* gene; Frigeni et al., 2017) or adults with secondary carnitine deficiencies due to chronic renal failure (Ames, 2010).

PPARα is crucially involved in energy and metabolic homeostasis. Fibrates (fibric acid derivatives, including fenofibrate and the enhanced medication-pemafibrate) are a class of PPARα agonists that lowers blood TAG through decreasing VLDL production by liver and promoting the removal of TAG from blood. Fibrates also moderately increase blood HDL cholesterol. Mechanistically, the PPARα agonists activate PPARα, promote peroxisomal and mitochondrial FAO, initiate cellular cascade to upregulate lipoprotein lipase, and ultimately cause more efficient catabolism of VLDL and TAG (Lakhia et al., 2018; Cheng et al., 2019; Yamashita et al., 2020).

CD36 mediates the internalization of lipids such as LCFAs, oxLDL, and oxidized phospholipid in both proximal tubule cells and podocytes. CD36 signaling is involved in FA-induced glomerular injury (Hua et al., 2015). The ApoA-I mimetic 5A peptide is a CD36 antagonist, which was shown to reduce glomerular injury and tubulointerstitial fibrosis in mouse CKD models of subtotal nephrectomy with angiotensin II infusion or unilateral ureteral obstruction (Souza et al., 2016). 5A peptide was shown to form HDL-like particles to promote ABCA1-dependent cholesterol efflux (Islam et al., 2018) and thus may effectively treat patients with cardiovascular disease.

The herbal alkaloid Berberine (BBR) is used as a supplemental medicine and has shown clinical benefit in reduction of LDL and TAG in diabetic and hypertensive patients (Koppen et al., 2017). BBR has wide spectrum pharmacological effects through its various action of mechanisms such as increasing LDL-receptor mediated hepatic clearance of LDL cholesterol (Wang et al., 2014), protection of lipid-induced apoptosis by promoting FAO in PTEC (Sun et al., 2018), supporting PGC1α-regulated mitochondrial energy homeostasis in CKD model of *db/db* mice and cultured podocytes (Qin et al., 2019a), and podocyte protection *via* inhibition of mitochondrial fission and dysfunction (Qin et al., 2019b).

Mitochondrial Bioenergetics and Biogenesis-Promoting Agents

Niacin (vitamin B-3) was the first identified lipid-lowering drug in patients at late 1950s and currently used as an adjunct therapy to help the control of cholesterol. Niacin, at pharmacological dose, increases circulating HDL level to improve cholesterol clearance in peripheral tissues and also changes the composition and metabolism of ApoA-I and ApoA-II (Shepherd et al., 1979). The HDL boost effect of niacin is through different molecular mechanisms. First, niacin stabilizes surface ABCA1 expression and ApoA-I lipidation. Second, niacin inhibits surface expression of the hepatic HDL receptor β-ATP synthase, and thus increases HDL blood availability (Zhang et al., 2008). Third, niacin inhibits the hepatic TAG biosynthesis enzyme "diacylglycerol acyltransferase-2 (DGAT2)" to reduce TAG synthesis and leads to the subsequent VLDL/LDL destabilization (Ganji et al., 2004). The mechanisms of DGAT inhibition and TAG metabolism are active research area as more pharmacological drugs designs centering on the two DGAT enzymes (DGAT1 and DGAT2), which apparently have distinct and overlapping functions (Chitraju et al., 2019). Niacin was later found to be an important precursor of cofactor NAD^+, which promotes SIRT/PGC-1α activity and thus modulates mitochondrial energy homeostasis, biogenesis, and lipid metabolism (Kirkland and Meyer-Ficca, 2018; Romani et al., 2019). Moreover, niacin provides vascular benefits through NAD^+/SIRT mediated mechanism during endothelial lipotoxicity (Hughes-Large et al., 2014).

The AMPK/SIRT/PGC-1α axis is crucial for mitochondrial biogenesis (Duann and Lin, 2017). Agents modulating this process include metformin. Metformin, the most commonly prescribed drug for the treatment of type 2 diabetes as a glucose-lowering and insulin-sensitizing agent, is a biguanide drug that also actives the energy sensor AMPK. In animal nephropathy models, several pathologies were observed including reduced phosphorylation of acetyl-CoA carboxylase (ACC), a target of AMPK and the major enzyme in the control of FAO rate; decreased expressions of CPT1 and enzymes in mitochondrial biogenesis; and increased lipid accumulation and expression of pro-inflammatory cytokines and tubulointerstitial fibrosis. Metformin reduces renal fibrosis by improving AMPK-mediated phosphorylation of ACC and FA energy metabolism (Lee et al., 2018).

Mitochondria-Targeted Anti-oxidants

Lipid-mediated mitochondrial oxidative stress is common in many kidney diseases. The selective mitochondria-targeted antioxidants, such as MitoQ and MitoTEMPO, have been developed to mitigate mitochondrial oxidative stress. These small molecule agents could be delivered and concentrated at mitochondria matrix to function as ROS scavenger (Kezic et al., 2016). They are chimeric molecules of a lipophilic cation triphenylphosphonium (TPP+) conjugated with an antioxidant moiety such as ubiquinone (MitoQ; Kelso et al., 2001) or piperidine nitroxides (TEMPOL and TEMPO; Trnka et al., 2008).

MitoTEMPO could be uptaken and accumulated in energized mitochondria matrix several 100-fold to modulate coenzyme Q (CoQ) pool within mitochondria (Trnka et al., 2008). In a diabetic *db/db* mouse model, 7-week of CoQ10 (0.1% in food) oral administration significantly reduced the levels of serum creatinine and blood glucose and albumin-to-creatinine ratio, in accordance with renal morphological restoration (Sun et al., 2019). CoQ10 ameliorates DN-induced mitochondrial dysfunction and oxidative stress through its activation of mitophagy-mediated glomerular mitochondria homeostasis both *in vivo* and *in vitro*. In this study, MitoTEMPO (3 mg/kg/day) restored mitophagy and alleviated kidney dysfunction in glomeruli of *db/db* mice in a similar manner as CoQ10 treatment (Sun et al., 2019).

In a mouse sub-total nephrectomy-induced renal fibrosis CKD model, MitoTEMPO rescued impaired renal function and alleviated renal fibrosis by reducing inflammation cytokines, mitochondrial dysfunction, ER stress, and profibrotic factors (Liu et al., 2018).

Additionally, in a clinically relevant murine model of abdominal sepsis (cecal ligation and puncture, CLP), a single delayed high dose of MitoTEMPO (10 mg/kg, given at 6 h post-CLP) could reverse renal mitochondrial dysfunction and attenuated sepsis-induced AKI by 18 h. MitoTEMPO decreased mitochondrial superoxide level, protected ETC respiration, improved renal microcirculation and glomerular filtration rate. Importantly, MitoTEMPO treatment significantly increased 96-h survival rate from 40% in untreated mice to 80% (Patil et al., 2014). The beneficial effect of MitoTEMPO is still under debate as it failed to exert long-term benefits in a later CLP-AKI study (Rademann et al., 2017). However, in a rat puromycin aminonucleoside (PAN)-induced glomerular damage model, a model mimicking children minimal-change nephrotic syndrome (MCNS), a 10-day MitoTEMPO treatment (1 day prior to PAN-injury and continued for 9 additional days) reduced the level of urinary protein, urinary lipid peroxidation and the expression of oxidative stress markers in glomeruli and plasma; although the overall renal function seemed not significantly improved as measure of creatinine clearance (Fujii et al., 2020). In summary, more research is warranted to validate renoprotective effects of MitoTEMPO.

MitoQ is a mitochondria targeted antioxidant of CoQ analogue, which could be accumulated in mitochondria up to 1,000-fold. In a type 1 monogenic diabetes of the young [MODY, the *Ins2Akita* (Akita)] mouse model, oral administration of MitoQ over a 12-week period prevented diabetic nephropathy (Chacko et al., 2010). MitoQ treatment did not alter the glycaemic status of diabetic animals. However, MitoQ significantly decreased urinary albumin levels in diabetic mice. MitoQ offered benefits in prevention of diabetes-induced tubular dysfunction and protection of glomerular function as measured by radioactive tracer clearance capacity. Moreover, MitoQ decreased pathogenic glomerular GBM thickening and reduced interstitial fibrosis through prevention of EMT (epithelial-to-mesenchymal transition) process in Akita mice (Chacko et al., 2010). Recently, in a diabetic *db/db* mouse model, Ward et al. confirmed the renoprotective effects of MitoQ treatment through daily intragastric gavage over a period of 12-week. MitoQ improved renal function, decreased glomerular hyperfiltration, albuminuria, and prevented interstitial fibrosis (Ward et al., 2017). In a mouse ischemia-reperfusion induced AKI (IRI) model, administration of MitoQ prior to the onset of ischemia was shown to reduce oxidative damage and severity of renal IRI (Dare et al., 2015). Despite the great success of mitochondria-targeting antioxidants in preclinical studies, their clinical effects on CKD patients remain to be verified. However, MitoQ supplementation was linked to restoration of endothelial function and reduces aortic arterial stiffness in aging humans, thus offers potential promise in vascular treatment in CKD patients (Rossman et al., 2018).

Cardiolipin-Targeting Peptides

In mice, a long-term (28 weeks) high fat diet (HFD) caused mitochondrial dysfunction and structural alterations, such as reduction in size and loss of matrix density and IMM cristae, in renal cells including proximal tubular cells, podocytes and glomerular endothelial cells. The mitochondrial injury led to ER stress, lipid droplets accumulation, autophagy, apoptosis, and subsequent inflammation, proteinuria, and fibrosis (Szeto et al., 2016). The mitochondrial injury could be due to loss and/or peroxidation of cardiolipin, the major structural and functional regulator of IMM cristae. Such mitochondrial injury could be prevented with cardiolipin-stabilizing tetrapeptide SS31 (namely, Elamipretide, MTP-13, or Bendavia), which reduces HFD-induced lipid accumulation, toxic ROS production, regulates cytochrome C activity, and restores AMPK signaling (Szeto et al., 2016; Szeto, 2017). The mitochondria protective effect of SS31 after ischemia-AKI prevents prolonged inflammation and arrests CKD transition (Szeto et al., 2017). Elamipretide is on a phase 2a clinical trial in patients with atherosclerotic renal artery stenosis during stent revascularization, with promising results (NCT01755858; Saad et al., 2017) and was shown to improve mitochondria function in the human failing heart (Chatfield et al., 2019). The clinical effects of Elamipretide on kidney disease, however, require further investigations.

CONCLUDING REMARKS

Mitochondria are the "powerhouse" of the high-energy demanding kidney cells. Crosstalk between mitochondria, nucleus, endoplasmic reticulum, and peroxisomes impacts numerous cellular functions. Mitochondrial bioenergetics, adaptation of energy metabolism, and mitochondrial biogenesis during physiology or stress conditions are tightly linked to body lipid homeostasis, as well as health and disease states of kidney. Dysfunctional mitochondria could lead to dyslipidemia, microvasculature damage, inflammation, kidney fibrosis, or even kidney failure. The evolving knowledge of the molecular mechanisms modulating mitochondrial energy homeostasis and lipid metabolism suggest that normalizing renal cell mitochondrial function and energy balance could be an important preventative strategy against dyslipidemia and could provide new drug targets in kidney diseases.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and drafting the work and critically reviewed and revised for the intellectual accuracy of the contents. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by an intramural Fund from The Ohio State University (to P-HL).

REFERENCES

Agrawal, S., Zaritsky, J. J., Fornoni, A., and Smoyer, W. E. (2017). Dyslipidaemia in nephrotic syndrome: mechanisms and treatment. *Nat. Rev. Nephrol.* 14:70. doi: 10.1038/nrneph.2017.175

Akhtar, S., and Siragy, H. M. (2019). Pro-renin receptor suppresses mitochondrial biogenesis and function via AMPK/SIRT-1/PGC-1alpha pathway in diabetic kidney. *PLoS One* 14:e0225728. doi: 10.1371/journal.pone.0225728

Ames, B. N. (2010). Optimal micronutrients delay mitochondrial decay and age-associated diseases. *Mech. Ageing Dev.* 131, 473–479. doi: 10.1016/j.mad.2010.04.005

Aon, M. A., Bhatt, N., and Cortassa, S. C. (2014). Mitochondrial and cellular mechanisms for managing lipid excess. *Front. Physiol.* 5:282. doi: 10.3389/fphys.2014.00282

Ashraf, S., Gee, H. Y., Woerner, S., Xie, L. X., Vega-Warner, V., Lovric, S., et al. (2013). ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. *J. Clin. Invest.* 123, 5179–5189. doi: 10.1172/JCI69000

Attman, P. O., and Alaupovic, P. (1990). Pathogenesis of hyperlipidemia in the nephrotic syndrome. *Am. J. Nephrol.* 10, 69–75. doi: 10.1159/000168197

Bhargava, P., and Schnellmann, R. G. (2017). Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.* 13, 629–646. doi: 10.1038/nrneph.2017.107

Birk, A. V., Chao, W. M., Bracken, C., Warren, J. D., and Szeto, H. H. (2014). Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. *Br. J. Pharmacol.* 171, 2017–2028. doi: 10.1111/bph.12468

Bonen, A., Luiken, J. J., Arumugam, Y., Glatz, J. F., and Tandon, N. N. (2000). Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J. Biol. Chem.* 275, 14501–14508. doi: 10.1074/jbc.275.19.14501

Bonventre, J. V., and Yang, L. (2011). Cellular pathophysiology of ischemic acute kidney injury. *J. Clin. Invest.* 121, 4210–4221. doi: 10.1172/JCI45161

Bremer, J. (1983). Carnitine-metabolism and functions. *Physiol. Rev.* 63, 1420–1480. doi: 10.1152/physrev.1983.63.4.1420

Brinkkoetter, P. T., Bork, T., Salou, S., Liang, W., Mizi, A., Özel, C., et al. (2019). Anaerobic glycolysis maintains the glomerular filtration barrier independent of mitochondrial metabolism and dynamics. *Cell Rep.* 27, 1551.e5–1566.e5. doi: 10.1016/j.celrep.2019.04.012

Brivet, M., Boutron, A., Slama, A., Costa, C., Thuillier, L., Demaugre, F., et al. (1999). Defects in activation and transport of fatty acids. *J. Inherit. Metab. Dis.* 22, 428–441. doi: 10.1023/A:1005552106301

Canto, C., and Auwerx, J. (2009). PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr. Opin. Lipidol.* 20, 98–105. doi: 10.1097/MOL.0b013e328328d0a4

Canto, C., Menzies, K. J., and Auwerx, J. (2015). NAD(+) metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab.* 22, 31–53. doi: 10.1016/j.cmet.2015.05.023

Cargill, K., and Sims-Lucas, S. (2020). Metabolic requirements of the nephron. *Pediatr. Nephrol.* 35, 1–8. doi: 10.1007/s00467-018-4157-2

Cavanaugh, C., and Perazella, M. A. (2019). Urine sediment examination in the diagnosis and management of kidney disease: core curriculum 2019. *Am. J. Kidney Dis.* 73, 258–272. doi: 10.1053/j.ajkd.2018.07.012

Chacko, B. K., Reily, C., Srivastava, A., Johnson, M. S., Ye, Y., Ulasova, E., et al. (2010). Prevention of diabetic nephropathy in Ins2(+/)$^{-}$(AkitaJ) mice by the mitochondria-targeted therapy MitoQ. *Biochem. J.* 432, 9–19. doi: 10.1042/BJ20100308

Chatfield, K. C., Sparagna, G. C., Chau, S., Phillips, E. K., Ambardekar, A. V., Aftab, M., et al. (2019). Elamipretide improves mitochondrial function in the failing human heart. *JACC Basic Transl. Sci.* 4, 147–157. doi: 10.1016/j.jacbts.2018.12.005

Chen, Y., He, L., Yang, Y., Chen, Y., Song, Y., Lu, X., et al. (2019). The inhibition of Nrf2 accelerates renal lipid deposition through suppressing the ACSL1 expression in obesity-related nephropathy. *Ren. Fail.* 41, 821–831. doi: 10.1080/0886022X.2019.1655450

Cheng, H. S., Tan, W. R., Low, Z. S., Marvalim, C., Lee, J. Y. H., and Tan, N. S. (2019). Exploration and development of PPAR modulators in health and disease: an update of clinical evidence. *Int. J. Mol. Sci.* 20:5055. doi: 10.3390/ijms20205055

Chitraju, C., Walther, T. C., and Farese, R. V. Jr. (2019). The triglyceride synthesis enzymes DGAT1 and DGAT2 have distinct and overlapping functions in adipocytes. *J. Lipid Res.* 60, 1112–1120. doi: 10.1194/jlr.M093112

Chung, K. W., Lee, E. K., Lee, M. K., Oh, G. T., Yu, B. P., and Chung, H. Y. (2018). Impairment of PPARalpha and the fatty acid oxidation pathway aggravates renal fibrosis during aging. *J. Am. Soc. Nephrol.* 29, 1223–1237. doi: 10.1681/ASN.2017070802

Cipolla, C. M., and Lodhi, I. J. (2017). Peroxisomal dysfunction in age-related diseases. *Trends Endocrinol. Metab.* 28, 297–308. doi: 10.1016/j.tem.2016.12.003

Corrales, P., Izquierdo-Lahuerta, A., and Medina-Gomez, G. (2018). Maintenance of kidney metabolic homeostasis by PPAR gamma. *Int. J. Mol. Sci.* 19:2063. doi: 10.3390/ijms19072063

Czumaj, A., Sledzinski, T., Carrero, J. J., Stepnowski, P., Sikorska-Wisniewska, M., Chmielewski, M., et al. (2019). Alterations of fatty acid profile may contribute to dyslipidemia in chronic kidney disease by influencing hepatocyte metabolism. *Int. J. Mol. Sci.* 20:2470. doi: 10.3390/ijms20102470

Dare, A. J., Bolton, E. A., Pettigrew, G. J., Bradley, J. A., Saeb-Parsy, K., and Murphy, M. P. (2015). Protection against renal ischemia-reperfusion injury in vivo by the mitochondria targeted antioxidant MitoQ. *Redox Biol.* 5, 163–168. doi: 10.1016/j.redox.2015.04.008

Du, X. G., and Ruan, X. Z. (2019). Lipid metabolism disorder and renal fibrosis. *Adv. Exp. Med. Biol.* 1165, 525–541. doi: 10.1007/978-981-13-8871-2_26

Duann, P., and Lin, P. H. (2017). Mitochondria damage and kidney disease. *Adv. Exp. Med. Biol.* 982, 529–551. doi: 10.1007/978-3-319-55330-6_27

Ducasa, G. M., Mitrofanova, A., and Fornoni, A. (2019a). Crosstalk between lipids and mitochondria in diabetic kidney disease. *Curr. Diab. Rep.* 19:144. doi: 10.1007/s11892-019-1263-x

Ducasa, G. M., Mitrofanova, A., Mallela, S. K., Liu, X., Molina, J., Sloan, A., et al. (2019b). ATP-binding cassette A1 deficiency causes cardiolipin-driven mitochondrial dysfunction in podocytes. *J. Clin. Invest.* 129, 3387–3400. doi: 10.1172/jci125316

Eirin, A., Lerman, A., and Lerman, L. O. (2017). The emerging role of mitochondrial targeting in kidney disease. *Handb. Exp. Pharmacol.* 240, 229–250. doi: 10.1007/164_2016_6

Ernster, L., and Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* 1271, 195–204. doi: 10.1016/0925-4439(95)00028-3

Escasany, E., Izquierdo-Lahuerta, A., and Medina-Gomez, G. (2019). Underlying mechanisms of renal lipotoxicity in obesity. *Nephron* 143, 28–32. doi: 10.1159/000494694

Ferro, C. J., Mark, P. B., Kanbay, M., Sarafidis, P., Heine, G. H., Rossignol, P., et al. (2018). Lipid management in patients with chronic kidney disease. *Nat. Rev. Nephrol.* 14, 727–749. doi: 10.1038/s41581-018-0072-9

Filippatos, T. D., Liontos, A., Christopoulou, E. C., and Elisaf, M. S. (2019). Novel hypolipidaemic drugs: mechanisms of action and main metabolic effects. *Curr. Vasc. Pharmacol.* 17, 332–340. doi: 10.2174/1570161116666180209112351

Flemming, N. B., Gallo, L. A., and Forbes, J. M. (2018). Mitochondrial dysfunction and signaling in diabetic kidney disease: oxidative stress and beyond. *Semin. Nephrol.* 38, 101–110. doi: 10.1016/j.semnephrol.2018.01.001

Florens, N., Calzada, C., Lyasko, E., Juillard, L., and Soulage, C. O. (2016). Modified lipids and lipoproteins in chronic kidney disease: a new class of uremic toxins. *Toxins (Basel)* 8:376. doi: 10.3390/toxins8120376

Fontecha-Barriuso, M., Martin-Sanchez, D., Martinez-Moreno, J. M., Monsalve, M., Ramos, A. M., Sanchez-Nino, M. D., et al. (2020). The role of PGC-1alpha and mitochondrial biogenesis in kidney diseases. *Biomolecules* 10:347. doi: 10.3390/biom10020347

Fornoni, A., Merscher, S., and Kopp, J. B. (2014). Lipid biology of the podocyte—new perspectives offer new opportunities. *Nat. Rev. Nephrol.* 10, 379–388. doi: 10.1038/nrneph.2014.87

Freeman, D. M., Chan, L., Yahaya, H., Holloway, P., and Ross, B. D. (1986). Magnetic resonance spectroscopy for the determination of renal metabolic rate in vivo. *Kidney Int.* 30, 35–42. doi: 10.1038/ki.1986.147

Frigeni, M., Balakrishnan, B., Yin, X., Calderon, F. R. O., Mao, R., Pasquali, M., et al. (2017). Functional and molecular studies in primary carnitine deficiency. *Hum. Mutat.* 38, 1684–1699. doi: 10.1002/humu.23315

Fujii, Y., Matsumura, H., Yamazaki, S., Shirasu, A., Nakakura, H., Ogihara, T., et al. (2020). Efficacy of a mitochondrion-targeting agent for reducing the

level of urinary protein in rats with puromycin aminonucleoside-induced minimal-change nephrotic syndrome. *PLoS One* 15:e0227414. doi: 10.1371/journal.pone.0227414

Gai, Z., Wang, T., Visentin, M., Kullak-Ublick, G. A., Fu, X., and Wang, Z. (2019). Lipid accumulation and chronic kidney disease. *Nutrients* 11:722. doi: 10.3390/nu11040722

Galvan, D. L., Green, N. H., and Danesh, F. R. (2017). The hallmarks of mitochondrial dysfunction in chronic kidney disease. *Kidney Int.* 92, 1051–1057. doi: 10.1016/j.kint.2017.05.034

Ganji, S. H., Tavintharan, S., Zhu, D., Xing, Y., Kamanna, V. S., and Kashyap, M. L. (2004). Niacin noncompetitively inhibits DGAT2 but not DGAT1 activity in HepG2 cells. *J. Lipid Res.* 45, 1835–1845. doi: 10.1194/jlr.M300403-JLR200

Ge, M., Fontanesi, F., Merscher, S., and Fornoni, A. (2020). The vicious cycle of renal lipotoxicity and mitochondrial dysfunction. *Front. Physiol.* 11:732. doi: 10.3389/fphys.2020.00732

Georgiou, D. K., Dagnino-Acosta, A., Lee, C. S., Griffin, D. M., Wang, H., Lagor, W. R., et al. (2015). $Ca2^{+}$ binding/permeation via calcium channel, CaV1.1, regulates the intracellular distribution of the fatty acid transport protein, CD36, and fatty acid metabolism. *J. Biol. Chem.* 290, 23751–23765. doi: 10.1074/jbc.M115.643544

Glatz, J. F., Nabben, M., Heather, L. C., Bonen, A., and Luiken, J. J. (2016). Regulation of the subcellular trafficking of CD36, a major determinant of cardiac fatty acid utilization. *Biochim. Biophys. Acta* 1861, 1461–1471. doi: 10.1016/j.bbalip.2016.04.008

Hager, M. R., Narla, A. D., and Tannock, L. R. (2017). Dyslipidemia in patients with chronic kidney disease. *Rev. Endocr. Metab. Disord.* 18, 29–40. doi: 10.1007/s11154-016-9402-z

Haunerland, N. H., and Spener, F. (2004). Fatty acid-binding proteins—insights from genetic manipulations. *Prog. Lipid Res.* 43, 328–349. doi: 10.1016/j.plipres.2004.05.001

Heine, G. H., Eller, K., Stadler, J. T., Rogacev, K. S., and Marsche, G. (2020). Lipid-modifying therapy in chronic kidney disease: pathophysiological and clinical considerations. *Pharmacol. Ther.* 207:107459. doi: 10.1016/j.pharmthera.2019.107459

Herman-Edelstein, M., Scherzer, P., Tobar, A., Levi, M., and Gafter, U. (2014). Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy. *J. Lipid Res.* 55, 561–572. doi: 10.1194/jlr.P040501

Hershberger, K. A., Martin, A. S., and Hirschey, M. D. (2017). Role of NAD(+) and mitochondrial sirtuins in cardiac and renal diseases. *Nat. Rev. Nephrol.* 13, 213–225. doi: 10.1038/nrneph.2017.5

Hoenig, M. P., and Zeidel, M. L. (2014). Homeostasis, the milieu interieur, and the wisdom of the nephron. *Clin. J. Am. Soc. Nephrol.* 9, 1272–1281. doi: 10.2215/cjn.08860813

Houten, S. M., Violante, S., Ventura, F. V., and Wanders, R. J. (2016). The biochemistry and physiology of mitochondrial fatty acid beta-oxidation and its genetic disorders. *Annu. Rev. Physiol.* 78, 23–44. doi: 10.1146/annurev-physiol-021115-105045

Hua, W., Huang, H. Z., Tan, L. T., Wan, J. M., Gui, H. B., Zhao, L., et al. (2015). CD36 mediated fatty acid-induced podocyte apoptosis via oxidative stress. *PLoS One* 10:e0127507. doi: 10.1371/journal.pone.0127507

Hughes-Large, J. M., Pang, D. K., Robson, D. L., Chan, P., Toma, J., and Borradaile, N. M. (2014). Niacin receptor activation improves human microvascular endothelial cell angiogenic function during lipotoxicity. *Atherosclerosis* 237, 696–704. doi: 10.1016/j.atherosclerosis.2014.10.090

Islam, R. M., Pourmousa, M., Sviridov, D., Gordon, S. M., Neufeld, E. B., Freeman, L. A., et al. (2018). Structural properties of apolipoprotein A-I mimetic peptides that promote ABCA1-dependent cholesterol efflux. *Sci. Rep.* 8:2956. doi: 10.1038/s41598-018-20965-2

Izquierdo-Lahuerta, A., Martínez-García, C., and Medina-Gómez, G. (2016). Lipotoxicity as a trigger factor of renal disease. *J. Nephrol.* 29, 603–610. doi: 10.1007/s40620-016-0278-5

Jager, S., Handschin, C., St-Pierre, J., and Spiegelman, B. M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12017–12022. doi: 10.1073/pnas.0705070104

Jang, H. S., Noh, M. R., Jung, E. M., Kim, W. Y., Southekal, S., Guda, C., et al. (2020a). Proximal tubule cyclophilin D regulates fatty acid oxidation in cisplatin-induced acute kidney injury. *Kidney Int.* 97, 327–339. doi: 10.1016/j.kint.2019.08.019

Jang, H. S., Noh, M. R., Kim, J., and Padanilam, B. J. (2020b). Defective mitochondrial fatty acid oxidation and lipotoxicity in kidney diseases. *Front. Med. (Lausanne)* 7:65. doi: 10.3389/fmed.2020.00065

Johnson, A. C., Stahl, A., and Zager, R. A. (2005). Triglyceride accumulation in injured renal tubular cells: alterations in both synthetic and catabolic pathways. *Kidney Int.* 67, 2196–2209. doi: 10.1111/j.1523-1755.2005.00325.x

Kang, H. M., Ahn, S. H., Choi, P., Ko, Y. A., Han, S. H., Chinga, F., et al. (2015). Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat. Med.* 21, 37–46. doi: 10.1038/nm.3762

Karwi, Q. G., Jorg, A. R., and Lopaschuk, G. D. (2019). Allosteric, transcriptional and post-translational control of mitochondrial energy metabolism. *Biochem. J.* 476, 1695–1712. doi: 10.1042/BCJ20180617

Karwi, Q. G., Uddin, G. M., Ho, K. L., and Lopaschuk, G. D. (2018). Loss of metabolic flexibility in the failing heart. *Front. Cardiovasc. Med.* 5:68. doi: 10.3389/fcvm.2018.00068

Katsyuba, E., Mottis, A., Zietak, M., De Franco, F., van der Velpen, V., Gariani, K., et al. (2018). De novo NAD(+) synthesis enhances mitochondrial function and improves health. *Nature* 563, 354–359. doi: 10.1038/s41586-018-0645-6

Kelso, G. F., Porteous, C. M., Coulter, C. V., Hughes, G., Porteous, W. K., Ledgerwood, E. C., et al. (2001). Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *J. Biol. Chem.* 276, 4588–4596. doi: 10.1074/jbc.M009093200

Kennedy, D. J., Chen, Y., Huang, W., Viterna, J., Liu, J., Westfall, K., et al. (2013). CD36 and Na/K-ATPase-alpha1 form a proinflammatory signaling loop in kidney. *Hypertension* 61, 216–224. doi: 10.1161/HYPERTENSIONAHA.112.198770

Kezic, A., Spasojevic, I., Lezaic, V., and Bajcetic, M. (2016). Mitochondria-targeted antioxidants: future perspectives in kidney ischemia reperfusion injury. *Oxid. Med. Cell. Longev.* 2016:2950503. doi: 10.1155/2016/2950503

Khan, S., Cabral, P. D., Schilling, W. P., Schmidt, Z. W., Uddin, A. N., Gingras, A., et al. (2018). Kidney proximal tubule lipoapoptosis is regulated by fatty acid transporter-2 (FATP2). *J. Am. Soc. Nephrol.* 29, 81–91. doi: 10.1681/asn.2017030314

Kirkland, J. B., and Meyer-Ficca, M. L. (2018). Niacin. *Adv. Food Nutr. Res.* 83, 83–149. doi: 10.1016/bs.afnr.2017.11.003

Konig, B., Koch, A., Giggel, K., Dordschbal, B., Eder, K., and Stangl, G. I. (2008). Monocarboxylate transporter (MCT)-1 is up-regulated by PPARalpha. *Biochim. Biophys. Acta* 1780, 899–904. doi: 10.1016/j.bbagen.2008.03.002

Koppen, L. M., Whitaker, A., Rosene, A., and Beckett, R. D. (2017). Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: a systematic review. *J. Evid. Based Complementary Altern. Med.* 22, 956–968. doi: 10.1177/2156587216687695

Kronenberg, F. (2018). HDL in CKD-the devil is in the detail. *J. Am. Soc. Nephrol.* 29, 1356–1371. doi: 10.1681/ASN.2017070798

Kruger, C., Nguyen, T. T., Breaux, C., Guillory, A., Mangelli, M., Fridianto, K. T., et al. (2019). Proximal tubular cell-specific ablation of carnitine acetyltransferase causes tubular disease and secondary glomerulosclerosis. *Diabetes* 68, 819–831. doi: 10.2337/db18-0090

Lakhia, R., Yheskel, M., Flaten, A., Quittner-Strom, E. B., Holland, W. L., and Patel, V. (2018). PPARalpha agonist fenofibrate enhances fatty acid beta-oxidation and attenuates polycystic kidney and liver disease in mice. *Am. J. Physiol. Ren. Physiol.* 314, F122–F131. doi: 10.1152/ajprenal.00352.2017

Lan, R., Geng, H., Singha, P. K., Saikumar, P., Bottinger, E. P., Weinberg, J. M., et al. (2016). Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. *J. Am. Soc. Nephrol.* 27, 3356–3367. doi: 10.1681/ASN.2015020177

Lee, M., Katerelos, M., Gleich, K., Galic, S., Kemp, B. E., Mount, P. F., et al. (2018). Phosphorylation of acetyl-CoA carboxylase by AMPK reduces renal fibrosis and is essential for the anti-fibrotic effect of metformin. *J. Am. Soc. Nephrol.* 29, 2326–2336. doi: 10.1681/ASN.2018010050

Li, S. Y., and Susztak, K. (2018). The role of peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) in kidney disease. *Semin. Nephrol.* 38, 121–126. doi: 10.1016/j.semnephrol.2018.01.003

Li, X., Zhang, T., Geng, J., Wu, Z., Xu, L., Liu, J., et al. (2019). Advanced oxidation protein products promote lipotoxicity and tubulointerstitial fibrosis via CD36/beta-catenin pathway in diabetic nephropathy. *Antioxid. Redox Signal.* 31, 521–538. doi: 10.1089/ars.2018.7634

Liu, Y., Wang, Y., Ding, W., and Wang, Y. (2018). Mito-TEMPO alleviates renal fibrosis by reducing inflammation, mitochondrial dysfunction, and

endoplasmic reticulum stress. *Oxid. Med. Cell. Longev.* 2018:5828120. doi: 10.1155/2018/5828120

Luiken, J. J., Chanda, D., Nabben, M., Neumann, D., and Glatz, J. F. (2016). Post-translational modifications of CD36 (SR-B2): implications for regulation of myocellular fatty acid uptake. *Biochim. Biophys. Acta* 1862, 2253–2258. doi: 10.1016/j.bbadis.2016.09.004

Lv, T., Hu, Y., Ma, Y., Zhen, J., Xin, W., and Wan, Q. (2019). GCN5L1 controls renal lipotoxicity through regulating acetylation of fatty acid oxidation enzymes. *J. Physiol. Biochem.* 75, 597–606. doi: 10.1007/s13105-019-00711-6

Maatman, R. G., Van Kuppevelt, T. H., and Veerkamp, J. H. (1991). Two types of fatty acid-binding protein in human kidney. Isolation, characterization and localization. *Biochem. J.* 273, 759–766. doi: 10.1042/bj2730759

Merscher, S., Pedigo, C. E., and Mendez, A. J. (2014). Metabolism, energetics, and lipid biology in the podocyte—cellular cholesterol-mediated glomerular injury. *Front. Endocrinol. (Lausanne)* 5:169. doi: 10.3389/fendo.2014.00169

Meyer, C., Nadkarni, V., Stumvoll, M., and Gerich, J. (1997). Human kidney free fatty acid and glucose uptake: evidence for a renal glucose-fatty acid cycle. *Am. J. Phys.* 273, E650–E654. doi: 10.1152/ajpendo.1997.273.3.E650

Møller, J. C., and Skriver, E. (1985). Quantitative ultrastructure of human proximal tubules and cortical interstitium in chronic renal disease (hydronephrosis). *Virchows Arch. A Pathol. Anat. Histopathol.* 406, 389–406. doi: 10.1007/BF00710231

Moorhead, J. F., Chan, M. K., El-Nahas, M., and Varghese, Z. (1982). Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* 2, 1309–1311. doi: 10.1016/s0140-6736(82)91513-6

Morigi, M., Perico, L., and Benigni, A. (2018). Sirtuins in renal health and disease. *J. Am. Soc. Nephrol.* 29, 1799–1809. doi: 10.1681/ASN.2017111218

Nishi, H., Higashihara, T., and Inagi, R. (2019). Lipotoxicity in kidney, heart, and skeletal muscle dysfunction. *Nutrients* 11:1664. doi: 10.3390/nu11071664

Nishi, H., and Nangaku, M. (2019). Podocyte lipotoxicity in diabetic kidney disease. *Kidney Int.* 96, 809–812. doi: 10.1016/j.kint.2019.07.013

Nosadini, R., and Tonolo, G. (2011). Role of oxidized low density lipoproteins and free fatty acids in the pathogenesis of glomerulopathy and tubulointerstitial lesions in type 2 diabetes. *Nutr. Metab. Cardiovasc. Dis.* 21, 79–85. doi: 10.1016/j.numecd.2010.10.002

Nsiah-Sefaa, A., and McKenzie, M. (2016). Combined defects in oxidative phosphorylation and fatty acid β-oxidation in mitochondrial disease. *Biosci. Rep.* 36:e00313. doi: 10.1042/BSR20150295

O'Brien, E. S., Nucci, N. V., Fuglestad, B., Tommos, C., and Wand, A. J. (2015). Defining the apoptotic trigger: the interaction of cytochrome C and cardiolipin. *J. Biol. Chem.* 290, 30879–30887. doi: 10.1074/jbc.M115.689406

Okamura, D. M., Lopez-Guisa, J. M., Koelsch, K., Collins, S., and Eddy, A. A. (2007). Atherogenic scavenger receptor modulation in the tubulointerstitium in response to chronic renal injury. *Am. J. Physiol. Ren. Physiol.* 293, F575–F585. doi: 10.1152/ajprenal.00063.2007

Okamura, D. M., Pennathur, S., Pasichnyk, K., Lopez-Guisa, J. M., Collins, S., Febbraio, M., et al. (2009). CD36 regulates oxidative stress and inflammation in hypercholesterolemic CKD. *J. Am. Soc. Nephrol.* 20, 495–505. doi: 10.1681/ASN.2008010009

Opazo-Ríos, L., Mas, S., Marín-Royo, G., Mezzano, S., Gómez-Guerrero, C., Moreno, J. A., et al. (2020). Lipotoxicity and diabetic nephropathy: novel mechanistic insights and therapeutic opportunities. *Int. J. Mol. Sci.* 21:2632. doi: 10.3390/ijms21072632

Ozaltin, F. (2014). Primary coenzyme Q10 (CoQ 10) deficiencies and related nephropathies. *Pediatr. Nephrol.* 29, 961–969. doi: 10.1007/s00467-013-2482-z

Patil, N. K., Parajuli, N., MacMillan-Crow, L. A., and Mayeux, P. R. (2014). Inactivation of renal mitochondrial respiratory complexes and manganese superoxide dismutase during sepsis: mitochondria-targeted antioxidant mitigates injury. *Am. J. Physiol. Ren. Physiol.* 306, F734–F743. doi: 10.1152/ajprenal.00643.2013

Pelsers, M. M. (2008). Fatty acid-binding protein as marker for renal injury. *Scand. J. Clin. Lab. Investig. Suppl.* 241, 73–77. doi: 10.1080/00365510802150133

Pennathur, S., Pasichnyk, K., Bahrami, N. M., Zeng, L., Febbraio, M., Yamaguchi, I., et al. (2015). The macrophage phagocytic receptor CD36 promotes fibrogenic pathways on removal of apoptotic cells during chronic kidney injury. *Am. J. Pathol.* 185, 2232–2245. doi: 10.1016/j.ajpath.2015.04.016

Pietrocola, F., Galluzzi, L., Bravo-San Pedro, J. M., Madeo, F., and Kroemer, G. (2015). Acetyl coenzyme A: a central metabolite and second messenger. *Cell Metab.* 21, 805–821. doi: 10.1016/j.cmet.2015.05.014

Portilla, D. (2003). Energy metabolism and cytotoxicity. *Semin. Nephrol.* 23, 432–438. doi: 10.1016/S0270-9295(03)00088-3

Poyan Mehr, A., Tran, M. T., Ralto, K. M., Leaf, D. E., Washco, V., Messmer, J., et al. (2018). De novo NAD(+) biosynthetic impairment in acute kidney injury in humans. *Nat. Med.* 24, 1351–1359. doi: 10.1038/s41591-018-0138-z

Qin, X., Jiang, M., Zhao, Y., Gong, J., Su, H., Yuan, F., et al. (2019a). Berberine protects against diabetic kidney disease via promoting PGC-1α-regulated mitochondrial energy homeostasis. *Br. J. Pharmacol.* 177, 3646–3661. doi: 10.1111/bph.14935

Qin, X., Zhao, Y., Gong, J., Huang, W., Su, H., Yuan, F., et al. (2019b). Berberine protects glomerular podocytes via inhibiting Drp1-mediated mitochondrial fission and dysfunction. *Theranostics* 9, 1698–1713. doi: 10.7150/thno.30640

Rademann, P., Weidinger, A., Drechsler, S., Meszaros, A., Zipperle, J., Jafarmadar, M., et al. (2017). Mitochondria-targeted antioxidants SkQ1 and MitoTEMPO failed to exert a long-term beneficial effect in murine polymicrobial sepsis. *Oxid. Med. Cell. Longev.* 2017:6412682. doi: 10.1155/2017/6412682

Rahman, E. U., Ruan, X. Z., Chana, R. S., Brunskill, N. J., Gaya, J., Powis, S. H., et al. (2008). Mesangial matrix-activated monocytes express functional scavenger receptors and accumulate intracellular lipid. *Nephrol. Dial. Transplant.* 23, 1876–1885. doi: 10.1093/ndt/gfm901

Ralto, K. M., Rhee, E. P., and Parikh, S. M. (2020). NAD(+) homeostasis in renal health and disease. *Nat. Rev. Nephrol.* 16, 99–111. doi: 10.1038/s41581-019-0216-6

Romani, M., Hofer, D. C., Katsyuba, E., and Auwerx, J. (2019). Niacin: an old lipid drug in a new NAD(+) dress. *J. Lipid Res.* 60, 741–746. doi: 10.1194/jlr.S092007

Rosenson, R. S., Hegele, R. A., and Koenig, W. (2019). Cholesterol-lowering agents. *Circ. Res.* 124, 364–385. doi: 10.1161/CIRCRESAHA.118.313238

Rossman, M. J., Santos-Parker, J. R., Steward, C. A. C., Bispham, N. Z., Cuevas, L. M., Rosenberg, H. L., et al. (2018). Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults. *Hypertension* 71, 1056–1063. doi: 10.1161/HYPERTENSIONAHA.117.10787

Ruan, X. Z., Varghese, Z., Powis, S. H., and Moorhead, J. F. (1999). Human mesangial cells express inducible macrophage scavenger receptor. *Kidney Int.* 56, 440–451. doi: 10.1046/j.1523-1755.1999.00587.x

Ruggiero, C., Elks, C. M., Kruger, C., Cleland, E., Addison, K., Noland, R. C., et al. (2014). Albumin-bound fatty acids but not albumin itself alter redox balance in tubular epithelial cells and induce a peroxide-mediated redox-sensitive apoptosis. *Am. J. Physiol. Ren. Physiol.* 306, F896–F906. doi: 10.1152/ajprenal.00484.2013

Rysz, J., Gluba-Brzozka, A., Rysz-Gorzynska, M., and Franczyk, B. (2020). The role and function of HDL in patients with chronic kidney disease and the risk of cardiovascular disease. *Int. J. Mol. Sci.* 21:601. doi: 10.3390/ijms21020601

Saad, A., Herrmann, S. M. S., Eirin, A., Ferguson, C. M., Glockner, J. F., Bjarnason, H., et al. (2017). Phase 2a clinical trial of mitochondrial protection (elamipretide) during stent revascularization in patients with atherosclerotic renal artery stenosis. *Circ. Cardiovasc. Interv.* 10:e005487. doi: 10.1161/CIRCINTERVENTIONS.117.005487

Sasaki, M., Yasuno, T., Ito, K., Matsunaga, A., Hisano, S., Abe, Y., et al. (2018). Focal segmental glomerulosclerosis with heterozygous apolipoprotein E5 (Glu3Lys). *CEN Case Rep.* 7, 225–228. doi: 10.1007/s13730-018-0331-4

Scerbo, D., Son, N. H., Sirwi, A., Zeng, L., Sas, K. M., Cifarelli, V., et al. (2017). Kidney triglyceride accumulation in the fasted mouse is dependent upon serum free fatty acids. *J. Lipid Res.* 58, 1132–1142. doi: 10.1194/jlr.M074427

Seok, S., Kim, Y. C., Byun, S., Choi, S., Xiao, Z., Iwamori, N., et al. (2018). Fasting-induced JMJD3 histone demethylase epigenetically activates mitochondrial fatty acid beta-oxidation. *J. Clin. Invest.* 128, 3144–3159. doi: 10.1172/JCI97736

Shepherd, J., Packard, C. J., Patsch, J. R., Gotto, A. M. Jr., and Taunton, O. D. (1979). Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein a metabolism. *J. Clin. Invest.* 63, 858–867. doi: 10.1172/JCI109385

Shi, L., and Tu, B. P. (2015). Acetyl-CoA and the regulation of metabolism: mechanisms and consequences. *Curr. Opin. Cell Biol.* 33, 125–131. doi: 10.1016/j.ceb.2015.02.003

Sieber, J., and Jehle, A. W. (2014). Free fatty acids and their metabolism affect function and survival of podocytes. *Front. Endocrinol. (Lausanne)* 5:186. doi: 10.3389/fendo.2014.00186

Simon, N., and Hertig, A. (2015). Alteration of fatty acid oxidation in tubular epithelial cells: from acute kidney injury to renal fibrogenesis. *Front. Med. (Lausanne)* 2:52. doi: 10.3389/fmed.2015.00052

Smith, B. K., Jain, S. S., Rimbaud, S., Dam, A., Quadrilatero, J., Ventura-Clapier, R., et al. (2011). FAT/CD36 is located on the outer mitochondrial membrane, upstream of long-chain acyl-CoA synthetase, and regulates palmitate oxidation. *Biochem. J.* 437, 125–134. doi: 10.1042/BJ20101861

Souza, A. C., Bocharov, A. V., Baranova, I. N., Vishnyakova, T. G., Huang, Y. G., Wilkins, K. J., et al. (2016). Antagonism of scavenger receptor CD36 by 5A peptide prevents chronic kidney disease progression in mice independent of blood pressure regulation. *Kidney Int.* 89, 809–822. doi: 10.1016/j.kint.2015.12.043

Stadler, K., Goldberg, I. J., and Susztak, K. (2015). The evolving understanding of the contribution of lipid metabolism to diabetic kidney disease. *Curr. Diab. Rep.* 15:40. doi: 10.1007/s11892-015-0611-8

Stallons, L. J., Funk, J. A., and Schnellmann, R. G. (2013). Mitochondrial homeostasis in acute organ failure. *Curr. Pathobiol. Rep.* 1, 169–177. doi: 10.1007/s40139-013-0023-x

Su, X., and Abumrad, N. A. (2009). Cellular fatty acid uptake: a pathway under construction. *Trends Endocrinol. Metab.* 20, 72–77. doi: 10.1016/j.tem.2008.11.001

Su, H., Wan, C., Lei, C. T., Zhang, C. Y., Ye, C., Tang, H., et al. (2017). Lipid deposition in kidney diseases: interplay among redox, lipid mediators, and renal impairment. *Antioxid. Redox Signal.* 28, 1027–1043. doi: 10.1089/ars.2017.7066

Sudhakaran, S., Bottiglieri, T., Tecson, K. M., Kluger, A. Y., and McCullough, P. A. (2018). Alteration of lipid metabolism in chronic kidney disease, the role of novel antihyperlipidemic agents, and future directions. *Rev. Cardiovasc. Med.* 19, 77–88. doi: 10.31083/j.rcm.2018.03.908

Sun, J., Chen, X., Liu, T., Jiang, X., Wu, Y., Yang, S., et al. (2018). Berberine protects against palmitate-induced apoptosis in tubular epithelial cells by promoting fatty acid oxidation. *Med. Sci. Monit.* 24, 1484–1492. doi: 10.12659/MSM.908927

Sun, J., Zhu, H., Wang, X., Gao, Q., Li, Z., and Huang, H. (2019). CoQ10 ameliorates mitochondrial dysfunction in diabetic nephropathy through mitophagy. *J. Endocrinol.* doi: 10.1530/JOE-18-0578 [Epub ahead of print]

Susztak, K., Ciccone, E., McCue, P., Sharma, K., and Bottinger, E. P. (2005). Multiple metabolic hits converge on CD36 as novel mediator of tubular epithelial apoptosis in diabetic nephropathy. *PLoS Med.* 2:e45. doi: 10.1371/journal.pmed.0020045

Szeto, H. H. (2017). Pharmacologic approaches to improve mitochondrial function in AKI and CKD. *J. Am. Soc. Nephrol.* 28, 2856–2865. doi: 10.1681/ASN.2017030247

Szeto, H. H., Liu, S., Soong, Y., Alam, N., Prusky, G. T., and Seshan, S. V. (2016). Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. *Kidney Int.* 90, 997–1011. doi: 10.1016/j.kint.2016.06.013

Szeto, H. H., Liu, S., Soong, Y., Seshan, S. V., Cohen-Gould, L., Manichev, V., et al. (2017). Mitochondria protection after acute ischemia prevents prolonged upregulation of IL-1β and IL-18 and arrests CKD. *J. Am. Soc. Nephrol.* 28, 1437–1449. doi: 10.1681/ASN.2016070761

Thapa, D., Wu, K., Stoner, M. W., Xie, B., Zhang, M., Manning, J. R., et al. (2018). The protein acetylase GCN5L1 modulates hepatic fatty acid oxidation activity via acetylation of the mitochondrial beta-oxidation enzyme HADHA. *J. Biol. Chem.* 293, 17676–17684. doi: 10.1074/jbc.AC118.005462

Thomas, S. R., Neuzil, J., and Stocker, R. (1996). Cosupplementation with coenzyme Q prevents the prooxidant effect of alpha-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. *Arterioscler. Thromb. Vasc. Biol.* 16, 687–696. doi: 10.1161/01.ATV.16.5.687

Thongnak, L., Pongchaidecha, A., and Lungkaphin, A. (2020). Renal lipid metabolism and lipotoxicity in diabetes. *Am. J. Med. Sci.* 359, 84–99. doi: 10.1016/j.amjms.2019.11.004

Tran, M. T., Zsengeller, Z. K., Berg, A. H., Khankin, E. V., Bhasin, M. K., Kim, W., et al. (2016). PGC1alpha drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature* 531, 528–532. doi: 10.1038/nature17184

Trnka, J., Blaikie, F. H., Smith, R. A., and Murphy, M. P. (2008). A mitochondria-targeted nitroxide is reduced to its hydroxylamine by ubiquinol in mitochondria. *Free Radic. Biol. Med.* 44, 1406–1419. doi: 10.1016/j.freeradbiomed.2007.12.036

Vamecq, J., Dessein, A. F., Fontaine, M., Briand, G., Porchet, N., Latruffe, N., et al. (2012). Mitochondrial dysfunction and lipid homeostasis. *Curr. Drug Metab.* 13, 1388–1400. doi: 10.2174/138920012803762792

Vaziri, N. D. (2006). Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. *Am. J. Physiol. Ren. Physiol.* 290, F262–F272. doi: 10.1152/ajprenal.00099.2005

Vaziri, N. D. (2016). Disorders of lipid metabolism in nephrotic syndrome: mechanisms and consequences. *Kidney Int.* 90, 41–52. doi: 10.1016/j.kint.2016.02.026

Verdin, E. (2015). NAD(+) in aging, metabolism, and neurodegeneration. *Science* 350, 1208–1213. doi: 10.1126/science.aac4854

Violante, S., Achetib, N., van Roermund, C. W. T., Hagen, J., Dodatko, T., Vaz, F. M., et al. (2019). Peroxisomes can oxidize medium- and long-chain fatty acids through a pathway involving ABCD3 and HSD17B4. *FASEB J.* 33, 4355–4364. doi: 10.1096/fj.201801498R

Wakino, S., Hasegawa, K., and Itoh, H. (2015). Sirtuin and metabolic kidney disease. *Kidney Int.* 88, 691–698. doi: 10.1038/ki.2015.157

Walther, T. C., Chung, J., and Farese, R. V. Jr. (2017). Lipid droplet biogenesis. *Annu. Rev. Cell Dev. Biol.* 33, 491–510. doi: 10.1146/annurev-cellbio-100616-060608

Wang, J., Hao, J. W., Wang, X., Guo, H., Sun, H. H., Lai, X. Y., et al. (2019). DHHC4 and DHHC5 facilitate fatty acid uptake by palmitoylating and targeting CD36 to the plasma membrane. *Cell Rep.* 26, 209.e5–221.e5. doi: 10.1016/j.celrep.2018.12.022

Wang, Z., Heshka, S., Gallagher, D., Boozer, C. N., Kotler, D. P., and Heymsfield, S. B. (2000). Resting energy expenditure-fat-free mass relationship: new insights provided by body composition modeling. *Am. J. Physiol. Endocrinol. Metab.* 279, E539–E545. doi: 10.1152/ajpendo.2000.279.3.E539

Wang, J., and Li, Y. (2019). CD36 tango in cancer: signaling pathways and functions. *Theranostics* 9, 4893–4908. doi: 10.7150/thno.36037

Wang, Y., Yi, X., Ghanam, K., Zhang, S., Zhao, T., and Zhu, X. (2014). Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption. *Metabolism* 63, 1167–1177. doi: 10.1016/j.metabol.2014.05.013

Ward, M. S., Flemming, N. B., Gallo, L. A., Fotheringham, A. K., McCarthy, D. A., Zhuang, A., et al. (2017). Targeted mitochondrial therapy using MitoQ shows equivalent renoprotection to angiotensin converting enzyme inhibition but no combined synergy in diabetes. *Sci. Rep.* 7:15190. doi: 10.1038/s41598-017-15589-x

Weinberg, J. M. (2011). Mitochondrial biogenesis in kidney disease. *J. Am. Soc. Nephrol.* 22, 431–436. doi: 10.1681/ASN.2010060643

Widmeier, E., Yu, S., Nag, A., Chung, Y. W., Nakayama, M., Fernández-Del-Río, L., et al. (2020). ADCK4 deficiency destabilizes the coenzyme Q complex, which is rescued by 2,4-Dihydroxybenzoic acid treatment. *J. Am. Soc. Nephrol.* 31, 1191–1211. doi: 10.1681/ASN.2019070756

Wu, J., Chen, L., Zhang, D., Huo, M., Zhang, X., Pu, D., et al. (2009). Peroxisome proliferator-activated receptors and renal diseases. *Front. Biosci. (Landmark Ed.)* 14, 995–1009. doi: 10.2741/3291

Yamamoto, T., Noiri, E., Ono, Y., Doi, K., Negishi, K., Kamijo, A., et al. (2007). Renal L-type fatty acid—binding protein in acute ischemic injury. *J. Am. Soc. Nephrol.* 18, 2894–2902. doi: 10.1681/ASN.2007010097

Yamamoto, T., Takabatake, Y., Minami, S., Sakai, S., Fujimura, R., Takahashi, A., et al. (2020). Eicosapentaenoic acid attenuates renal lipotoxicity by restoring autophagic flux. *Autophagy*, 1–14. doi: 10.1080/15548627.2020.1782034

Yamamoto, T., Takabatake, Y., Takahashi, A., Kimura, T., Namba, T., Matsuda, J., et al. (2017). High-fat diet-induced lysosomal dysfunction and impaired autophagic flux contribute to lipotoxicity in the kidney. *J. Am. Soc. Nephrol.* 28, 1534–1551. doi: 10.1681/ASN.2016070731

Yamamoto, S., Yancey, P. G., Ikizler, T. A., Jerome, W. G., Kaseda, R., Cox, B., et al. (2012). Dysfunctional high-density lipoprotein in patients on chronic hemodialysis. *J. Am. Coll. Cardiol.* 60, 2372–2379. doi: 10.1016/j.jacc.2012.09.013

Yamashita, S., Masuda, D., and Matsuzawa, Y. (2020). Pemafibrate, a new selective PPARalpha modulator: drug concept and its clinical applications for dyslipidemia and metabolic diseases. *Curr. Atheroscler. Rep.* 22:5. doi: 10.1007/s11883-020-0823-5

Yang, X., Okamura, D. M., Lu, X., Chen, Y., Moorhead, J., Varghese, Z., et al. (2017). CD36 in chronic kidney disease: novel insights and therapeutic opportunities. *Nat. Rev. Nephrol.* 13, 769–781. doi: 10.1038/nrneph.2017.126

Yu, A. S. L., Chertow, G. M., Luyckx, V., Marsden, P. A., Skorecki, K., and Taal, M. W. (2019). *Brenner and Rector's the kidney*. *11th Edn*. *Vol. 2*. Philadelphia: Elsevier.

Zager, R. A., Johnson, A. C., and Becker, K. (2011). Acute unilateral ischemic renal injury induces progressive renal inflammation, lipid accumulation, histone modification, and "end-stage" kidney disease. *Am. J. Physiol. Ren. Physiol.* 301, F1334–F1345. doi: 10.1152/ajprenal.00431.2011

Zhang, L. H., Kamanna, V. S., Zhang, M. C., and Kashyap, M. L. (2008). Niacin inhibits surface expression of ATP synthase beta chain in HepG2 cells: implications for raising HDL. *J. Lipid Res.* 49, 1195–1201. doi: 10.1194/jlr.M700426-JLR200

Zhao, H. L., Sui, Y., Guan, J., He, L., Zhu, X., Fan, R. R., et al. (2008). Fat redistribution and adipocyte transformation in uninephrectomized rats. *Kidney Int.* 74, 467–477. doi: 10.1038/ki.2008.195

Zoja, C., Benigni, A., and Remuzzi, G. (2014). The Nrf2 pathway in the progression of renal disease. *Nephrol. Dial. Transplant.* 29, i19–i24. doi: 10.1093/ndt/gft224

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 04 September 2020
doi: 10.3389/fphys.2020.565023

Mitochondrial Dysfunction in Kidney Disease and Uremic Sarcopenia

*Koji Takemura[1], Hiroshi Nishi[1] and Reiko Inagi[2]**

[1] Division of Nephrology and Endocrinology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [2] Division of CKD Pathophysiology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Recently, there has been an increased focus on the influences of mitochondrial dysfunction on various pathologies. Mitochondria are major intracellular organelles with a variety of critical roles, such as adenosine triphosphate production, metabolic modulation, generation of reactive oxygen species, maintenance of intracellular calcium homeostasis, and the regulation of apoptosis. Moreover, mitochondria are attracting attention as a therapeutic target in several diseases. Additionally, a lot of existing agents have been found to have pharmacological effects on mitochondria. This review provides an overview of the mitochondrial change in the kidney and skeletal muscle, which is often complicated with sarcopenia and chronic kidney disease (CKD). Furthermore, the pharmacological effects of therapeutics for CKD on mitochondria are explored.

Keywords: mitochondria, CKD - chronic kidney disease, sarcopenia, kidney, skeletal muscle

OPEN ACCESS

Edited by:
Egor Plotnikov, Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russia

Reviewed by:
Jorge L. Gamboa, Vanderbilt University Medical Center, United States
Hirotaka Komaba, School of Medicine, Tokai University, Japan
Gaia Gherardi, University of Padua, Italy

****Correspondence:***
Reiko Inagi
inagi-npr@umin.ac.jp

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *23 May 2020*
Accepted: *12 August 2020*
Published: *04 September 2020*

Citation:
Takemura K, Nishi H and Inagi R (2020) Mitochondrial Dysfunction in Kidney Disease and Uremic Sarcopenia. Front. Physiol. 11:565023. doi: 10.3389/fphys.2020.565023

INTRODUCTION

Mitochondria originated approximately 1.5 billion years ago from α-proteobacterium via symbiosis within an ancestral eukaryotic host cell. Although mitochondria contain double membrane and serve as the main producer of adenosine triphosphate (ATP), their form and composition have evolved, and these organelles have gained a myriad of additional functions. This article reviews mitochondrial functions, the changes of these organelles in the kidney (**Figure 1A**) and skeletal muscle tissues (**Figure 1B**) in kidney diseases, and the potential effects of therapeutic agents on the mitochondria in treating kidney diseases and uremic sarcopenia.

MITOCHONDRIA BIOLOGY IN THE KIDNEY

Mitochondrial Structure

Mitochondria are intracellular organelles found in all eukaryotes. The mitochondrial structure contains a membrane structure of outer and inner mitochondria membrane (IMM) layers, with one compartment between the intermembrane space and the inner matrix. The mitochondrial outer mitochondria membrane (OMM) contains a porin that controls the transport of proteins into mitochondria and allows non-selective permeation of small molecule substances of approximately 1,550 kDa. The IMM contains a complex folded structure, the cristae, that contributes to oxidative phosphorylation to produce ATP, which is an energy source in the cell.

The renal proximal tubules contain more mitochondria than any other compartments in the kidney. Renal proximal tubules absorb more than 65% of the filtrate that passes through the glomerular membrane filter, such as glucose, ions, and albumin (Rector, 1983). Mitochondria often undergo transformation in both physiological and pathological conditions (Wakabayashi, 2002).

In a diseased kidney, premorse and gigantic mitochondria are noted in the cytoplasm of the proximal epithelial cells, as shown in early studies of human kidney samples using electron microscopy (Suzuki et al., 1975). The experimental literature also supports that mitochondria amounts largely diminish and their structure appears altered after acute kidney injury (AKI) (Lan et al., 2016). Although the molecular mechanism underlying the morphologic alteration has yet to be investigated, mitochondria represent one of the more vulnerable organelles for various types of toxic and pathogenic insults.

Mitochondrial Chromosome, Genome, DNA

Mitochondrial Biogenesis

The mitochondrial mass is increased through the cellular process called mitochondrial biogenesis to adapt to the ever-changing energy demand. Mitochondrial biogenesis leads to a greater mitochondrial metabolic capacity by increasing synthesis of metabolic enzymes. While the majority of mitochondrial molecules are encoded by the cell nuclear genes, most parts of the electron transport chain (ETC) that function as an energy generator are produced from mitochondrial genes. Mitochondrial DNA (mtDNA) is transcribed by the mitochondrial DNA-directed RNA polymerase, POLRMT (Graziewicz et al., 2006) and the essential enhancer is the mitochondrial transcription factor A (TFAM), which ensures mtRNA unwinding and flexing required for the POLRMT binding to the promoters. The expression of TFAM is regulated by nuclear respiratory factor 1 (Nrf1) binding to the specific promoter sites (Virbasius and Scarpulla, 1994; Gureev et al., 2019), suggesting that Nrf1 may be involved the biogenesis and energy production in the mitochondria (van Tienen et al., 2010; Benner et al., 2013). Also, researchers have discovered that peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) in brown adipocytes is a contributing factor for cold-mediated mitochondrial biogenesis (Puigserver et al., 1998). This transcription coactivator promotes the transcription of Nrf1 although PGC-1α acts as a coactivator for numerous genes including Nrf2 (Wu et al., 1999). PGC-1α also activates mitochondrial biogenesis in skeletal muscle and enhances the slow-twitch manifestations of the skeletal muscle, such as fatty acid oxidation and an increase in type I myosin heavy chain (Wu et al., 1999; Jornayvaz and Shulman, 2010). Furthermore, this coactivator is important for skeletal muscle remodeling in physical exercise (Lira et al., 2010).

In sepsis-associated AKI, the mitochondrial function deteriorates, and the expression of genes involved in oxidative phosphorylation is reduced (Parikh et al., 2015). Particularly, PGC-1α expression decreases as renal function declines, and renal function was impaired due to prolonged sepsis. The activation of PGC-1α may promote recovery from AKI caused by sepsis, and this application in therapy is expected. Recently, our laboratory revealed that mtDNA copy number and PGC-1α expression were reduced in the kidneys of animals with polycystic kidney disease (Ishimoto et al., 2017). Moreover, the eradication of mitochondrion-specific oxidants reduced intracellular superoxide and halted the proliferation of cyst epithelial cells via extracellular signal-related kinase inactivation (Ishimoto et al., 2017).

Mitochondrial DNA Leakage

In cells infected with pathogens (e.g., DNA viruses), the pathogen-derived double-stranded DNA appears in the cytoplasm. Cyclic GMP–AMP synthase (cGAS) is a pattern recognition receptor that recognizes double-stranded DNA in the cytoplasm and then binds to the trans-membrane protein, a stimulator of interferon genes (STING) localized on the endoplasmic reticulum (ER). Eventually, this reaction induces a type I interferon-mediated host defense response to DNA viruses (Chen et al., 2016). Moreover, the cGAS–STING pathway activation is involved in autoimmune and inflammatory reactions, likely resulting from the activation of cGAS by self-genomic DNA damage (Li and Chen, 2018).

Our group recently clarified the relationship between mitochondrial damage and the induction of cGAS–STING pathway in inflamed proximal tubular cells. In cisplatin-induced AKI, the cGAS–STING pathway was activated in the kidney (Maekawa et al., 2019). In STING-deficient mice, cisplatin-induced renal dysfunction and inflammatory responses were reduced. Additionally, the mitochondrial membrane potential was reduced in renal proximal tubular cells stimulated with cisplatin, and mtDNA leaked into the cytosol, thereby causing the activation of the cGAS–STING pathway (Maekawa et al., 2019). The inhibition of this pathway is a promising target for future treatments of AKI.

Mitochondrial Dynamics

Although mitochondrial biogenesis promotes new mitochondria production, mitochondria cannot be generated *de novo*. Instead, mitochondria form a dynamic network that can alter the shape and size and also add new content to pre-existing mitochondria. In other words, mitochondria actively and frequently undergo fusion and division (fission), such that long and extended structures are formed through fusion, and small fragmentation is induced through the division. Mitochondrial fusion is caused by several guanosine triphosphate hydrolases (GTPase), including mitofusin 1 (Mfn1), Mfn2, and optic atrophy 1 (Opa1). Both Mfn1 and Mfn2 are considered to contribute to OMM fusion, whereas Opa1 splicing contributes to IMM fusion. Conversely, mitochondrial fission is promoted by fission 1 protein (Fis1), that is localized on the OMM, and the GTPase, dynamin-related protein (Drp1). The mutation of Drp1 is associated with lethal neonatal defects in humans (Waterham et al., 2007), and the mice deficient in Drp1 are also embryonic lethal, whereas brain-specific Drp1 ablation causes developmental defects to the cerebellum (Wakabayashi et al., 2009). These findings suggest that mitochondrial fission plays an essential role in early development and differentiation.

In ischemic and cisplatin nephrotoxic AKI, these mitochondrial dynamics have been analyzed mainly in proximal tubules that are dependent on oxidative phosphorylation for the large demand of ATP necessary for solute transportation.

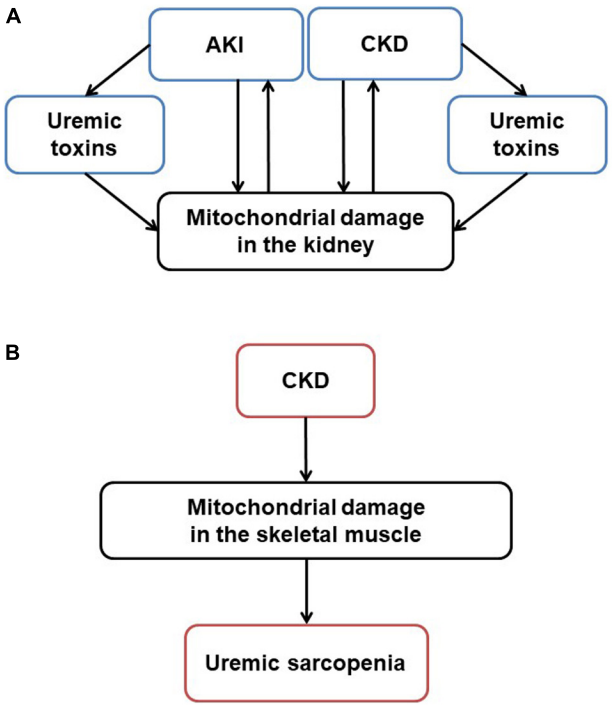


FIGURE 1 | Mitochondrial damage in CKD and uremic sarcopenia. **(A)** Stressors like sepsis, ischemia and toxins induce acute mitochondrial damage. Pathogenesis such as hypertension, diabetes, and obesity induce chronic mitochondrial damage. Uremic toxins accumulated in AKI and CKD also induce mitochondrial damage. On the other hand, mitochondrial damage itself exacerbates kidney damage, forming a vicious cycle in CKD progression. **(B)** In CKD, mitochondrial damage mediates uremic sarcopenia. AKI, acute kidney injury; CKD, chronic kidney disease; ROS, reactive oxygen species; ATP, adenosine triphosphate.

Mitochondrial fission initiated by Drp1 translocation to the OMM is often observable immediately after the injury (Brooks et al., 2009). Intriguingly, treatment with a small molecule that inhibits Drp1, Mdivi1, attenuated both tubular cell apoptosis and tubular tissue damage. Also, in cultured tubular cells, the silencing of *Drp1* or a dominant-negative Drp1 induction led to mitochondrial fragmentation and subsequent apoptosis. This concept of Drp1 to mediate not only mitochondrial fission but also subsequent ischemic renal tissue damage was further supported by the murine genetic deletion of *Drp1* in the proximal tubule epithelium (Perry et al., 2018). They also showed that delayed deletion of Drp1 in the recovery period after ischemic-reperfusion injury (IRI) resulted in improved kidney recovery and reduced fibrosis, implying that tubular mitochondrial fission and fusion might play a role in progression of AKI to fibrosis. Moreover, Drp1 phosphorylation is also implicated in diabetic renal tubular cells (Zhan et al., 2015) and podocytes (Ayanga et al., 2016). The deletion of Drp1 selectively in podocytes of *db/db* diabetic mice leads to an attenuated diabetic phenotype, such as seen through excessive oxygen consumption (Ayanga et al., 2016).

Mitochondria and ER Crosstalk

Eukaryotic cells contain various organelles besides the mitochondria. The functions of these individual organelles and the communication between them are essential for cell survival, proliferation, and differentiation. Intracellular transfer of vesicles enables communication between distant organelles via microtubules and actin along the cytoskeleton (Allan and Schroer, 1999; Bonifacino and Glick, 2004). Moreover, different organelles can also communicate with each other through direct contact and proximity signaling. The ER is located at the center of the membrane contact site (MCS) between organelles. Particularly, mitochondria-associated membranes (MAMs) have been intensively studied as a type of MCS formed through the ER (Fujimoto and Hayashi, 2011; Inoue et al., 2019). This machinery forms a raft-like structure rich in cholesterol and sphingolipids similar to caveola (Hayashi and Su, 2007) and enables molecular communications between ER and mitochondria via calcium, lipid synthases, inositol trisphosphate (IP3) receptors, and sarco/endoplasmic reticulum calcium-ATPases that are abundant and active on the MAM (Appenzeller-Herzog and Simmen, 2016). Notably, mitochondrial fission-promoting enzyme Mfn2 is located both in the mitochondria and partially in the ER. Furthermore, Mfn2 on the ER assembles a molecular complex with Mfn1 on the mitochondria that tightens the connection between ER and mitochondria (de Brito and Scorrano, 2008).

In the murine diabetic kidney, the MAM is significantly reduced and resembles the severity of tissue damage (Yang et al., 2019). Cultured tubular cells overexpressing the MAM-uncoupling protein (UCP), FATE-1, are resistant to high-glucose stimuli and have less cellular apoptosis (Yang et al., 2019). Although the mitochondrial-ER association has only lately been highlighted (Inoue et al., 2019; Yang et al., 2020), how this organelle crosstalk is altered in AKI or chronic kidney disease (CKD) remains largely unknown.

Energetics

Glycolysis and Tricarboxylic Acid (TCA) Cycle

Glycolysis is considered the most primitive metabolic system. This process catabolizes glucose into organic acids, such as pyruvate, and produces energy for the organism to consume. Under aerobic conditions, pyruvate produced by glycolysis is transported from the cytosol to the mitochondrial matrix through an active transporter. It is next decarboxylated by the pyruvate dehydrogenase complex (PDHC) and then converted to acetyl-CoA (Semenza, 2011; Gray et al., 2014). The acetyl-CoA enters the TCA cycle, which oxidizes acetyl-CoA to produce carbon dioxide. TCA produces coenzymes as three molecules of NADH, one molecule of $FADH_2$, GTP. Although carbon dioxide is excreted outside the mitochondria and no ATP is produced in the TCA cycle, the resulting coenzymes aide ATP production in the subsequent oxidative phosphorylation.

In ischemic AKI, glucose metabolism is differently altered in the cortex and the medulla of the kidney. Metabolites pyruvate and lactate are decreased in the cortex during early onset of AKI, while the TCA intermediates succinate and malate

are almost unchanged (Wei et al., 2014). These observations suggest a transient decrease in the use of glucose for energy metabolism in the cortex. By contrast, the kidney medulla showed a slower decrease in glycolysis and TCA cycle activity over time. Tubular cell glucose uptake and lactate production were accelerated in fibrotic kidney resembling human CKD, implying an enhancement of aerobic glycolysis flux (Ding et al., 2017). Interestingly, induction of aerobic glycolysis led to myofibroblast activation *in vitro*. Past research has illustrated that the metabolism of the renal medulla is primarily glycolysis, whereas aerobic oxidation of substrates and gluconeogenesis is prioritized in the renal cortex (Lee et al., 1962; Lee and Peterhm, 1969). This layer-dependent renal energy preference is explained by local tissue oxygen pressure. Moreover, in the outer medulla, where slightly less oxygen is available than in the cortex, glucose fuels the mitochondria (Eveloff et al., 1980), although succinate is prioritized as an energy source (Baverel et al., 1980). In the inner medulla, glycolysis plays a more critical role in metabolizing glucose to produce energy as oxygen consumption is lower (Baverel et al., 1980).

Fatty Acid Transport and β-Oxidation

Most fatty acids act as energy sources for peripheral tissues once they are released into the blood through the hydrolysis of triacylglycerols in adipose tissue. Circulating triacylglycerol is degraded by lipase to produce fatty acids and glycerin. Cytosolic fatty acids are converted to acyl-CoA by acyl-CoA synthetase, which cannot pass through the IMM of the mitochondria. First, the fatty acid portion of acyl-CoA is transferred to carnitine through carnitine acyltransferase to provide acylcarnitine. Then, acylcarnitine enters the mitochondrial matrix and reacts with Coenzyme A (CoA) and returns to acyl-CoA (Bremer, 1983; Brady et al., 1993). Acyl-CoA is then decomposed into two carbon units to produce acetyl-CoA in the stepwise sequence of oxidation, hydration, oxidation, and thiol cleavage. This process, known as β-oxidation, generates ubiquinol (QH2), NADH, GTP, and a sufficient amount of ATP (Raskind and El-Chaar, 2000).

As previously mentioned, glucose is a poor energy source for respiration in the kidney cortex, which contains glomeruli and proximal tubules. The preferred fuels are short- and long-chain fatty acids and endogenous lipids, as well as ketone bodies, lactate, and some amino acids (Weidemann and Krebs, 1969; Klein et al., 1980). Conversely, chronic hyperinsulinemia enhances degradation of triglycerides in the adipocytes, thus elevating serum levels of non-esterified fatty acids. These elevated levels lead to the ectopic accumulation of lipids in organs outside of the lipid tissue, including the kidney. The excessive accumulation of lipids results in cellular damage, known as lipotoxicity (Weinberg, 2006; Ertunc and Hotamisligil, 2016; Nishi et al., 2019). For instance, fatty acids accumulated in the mitochondrial matrix are vulnerable to lipid peroxidation (Schrauwen and Hesselink, 2004; Schrauwen et al., 2010; Sergi et al., 2019), which can have lipotoxic effects on DNA, RNA, and proteins of the mitochondrial machinery, leading to organelle dysfunction. In AKI and diabetic nephropathy, β-oxidation in the mitochondria is decreased and the formation of lipid droplets inside the cell are increased, resulting in diminished ATP production (Simon and Hertig, 2015). Stimulation of proximal tubular culture cells with the fatty acid palmitate invokes the accumulation of abnormal organelles because of poor acidification of lysosomes (Yamamoto et al., 2017), even promoting lipoapoptosis (Katsoulieris et al., 2010).

Electron Transfer System and Mitochondrial Reactive Oxygen Species

The ETC is responsible for mitochondrial oxidative phosphorylation, which produces ATP using the oxygen-based fatty acids and pyruvate. In this process, five electron-transporting enzyme complexes (complexes I to V) on the IMM, as well as the electron transporters ubiquinone and cytochrome c, excrete protons into the intermembrane space to drive ATP synthase. Although this process is efficient, insufficient reactions can produce reactive oxygen species (ROS). Oxidative stress reduces mitochondrial and cellular function and can even cause cell death by promoting lipid peroxidation in the IMM. This lipid peroxidation can change the membrane permeability and structure (Stewart and Heales, 2003) or disrupting calcium homeostasis, particularly affecting the oxidation state of specific thiol groups in proteins (Toescu, 2005). Mitochondria possess defense systems to scavenge ROS to protect them from excess radicals. For example, superoxide dismutase 2 (SOD2) transforms superoxide anions to oxygen and hydrogen peroxide (Weisiger and Fridovich, 1973). Also, coenzyme Q10 (CoQ10) can exist in two forms: Ubiquinone, an oxidized form, that acts as an electron carrier during mitochondrial respiration; and Ubiquinol, a reduced form, that is an endogenous antioxidant (Crane, 2001). Mutations in the genes that encode the CoQ10 pathway confer an inherited mitochondriopathy with primary renal involvement (Diomedi-Camassei et al., 2007).

In chronic hypoxic kidneys of rat, proteomic analysis identified maladaptive suppression of Cu/Zn-SOD enzymes as a mediator of a cycle of oxidative stress and subsequent renal injury (Son et al., 2008). Also, mice with cisplatin-induced AKI treated with CoQ10 has less depletion of their antioxidant defense mechanisms (glutathione level and SOD activity), lipid peroxidation, and renal tissue damage (Fouad et al., 2010). The significance of mitochondrial ROS levels has also been implicated in diabetic kidney diseases (DKD). Mitochondrial superoxide and ATP production were increased in type 2 diabetic *db/db* mice in the renal cortex, compared to control mice (Sourris et al., 2012); however, the excessive renal mitochondrial hydrogen peroxide production and membrane potential seen in *db/db* mice were attenuated with CoQ10 treatment. This normalization of mitochondrial ROS generation caused by CoQ10 treatment decreases albuminuria (Sourris et al., 2012). NADPH oxidases (NOX) are the principal enzymes to generate nitrogen species and ROS, with NOX4 localized within the renal cortex and upregulated under hyperglycemic conditions within the mitochondria (Block et al., 2009). Intriguingly, pharmacological inhibition of NOX4 has potential for treatment of renal histopathology and albuminuria in *db/db* mice (Sedeek et al., 2013). Also, chemical inactivation of NOX4 protected ApoE-deficient mice treated

with streptozotocin from both structural and functional kidney damage (Jha et al., 2014).

Electrochemical Gradient and Uncoupling

The pumping of protons into the transmembrane space results in a difference of proton concentrations between the inside and outside the IMM (i.e., electrochemical gradient). These protons can return to the matrix through the ATP synthase pump, at which uses the potential to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi). The generated ATP is then transported from the mitochondria to the cytoplasm by ATP/ADP transporters and becomes the active energy source for cells. Alternatively, proton in the mitochondrial intermembrane space released by the ETC may also return to the matrix by diffusing through the IMM without involvement in ATP synthesis. This flux is known as uncoupling, and the accumulated electrochemical potential is squandered as heat (Dlaskova et al., 2006). The transporter protein UCP uses this proton gradient between membranes to generate energy for oxidative phosphorylation (Ricquier et al., 1991).

The UCP has isoforms in mammals (UCP1–5). Thermogenin (UCP1) is present only in brown adipocytes, and contributes to heat production without movement during hibernation (Palou et al., 1998). Recently, UCP1 expression was reported in the ischemic kidneys (Jia et al., 2019) and speculated to protect the organ from hypoxia, since deletion of *UCP1* worsened both ischemia or cisplatin induced AKI. Furthermore, peroxisome proliferator-activator receptor (PPAR) γ agonist treatment increased UCP1 expression, suggesting their close relationship. Elsewhere, an early work combined immunohistochemistry and polymerase chain reaction techniques to unravel the UCP2 expression in rat kidneys, specifically the epithelial cells of the proximal tubules and the medullary thick ascending loop of Henle (Balaban and Mandel, 1980). Multiple reports suggest that tubular UCP2 expression is enhanced in kidneys that are diseased. Higher expression of UCP2 is found in diabetic kidneys (Balaban and Mandel, 1980). It was demonstrated that glutamate-stimulated oxygen consumption was increased in the isolated mitochondria from diabetic animals, and could be reduced by adding guanosine diphosphate, which inhibits UCP activity. These results imply that those mitochondria have increased uncoupling due to increased UCP2 protein expression (Friederich et al., 2008). In fibrotic kidneys, the expression of UCP2 in proximal tubular epithelial cells was increased (Jiang et al., 2013), and mice deficient in UCP2 were protected from kidney fibrosis induced by unilateral ureter obstruction (UUO). Intriguingly, UCP2 causes cultured epithelial cells to transdifferentiate and release cellular matrix. Regarding other UCP isoforms, UCP3 expression is found in the epithelial cells of the renal cell carcinoma (Braun et al., 2015). Intriguingly, their loss-of-function study indicates that UCP3 in carcinoma that originated from the proximal tubular cells helps resist against hypoxia/reoxygenation injury of cancer.

Calcium Storage

Intracellular calcium concentration is tightly regulated and plays a vital role in the signal transduction of cells. Although the ER stores the highest amount of intracellular calcium, mitochondria also have temporary capacity to store calcium, which contributes to the homeostasis of the overall intracellular calcium concentration (Brookes et al., 2004).

In kidney diseases, mitochondrial calcium uptake is impaired in tubular epithelia cells. Cation uptake in tubular cells is reduced per the cytoplasmic glutathione level in cisplatin-induced kidney damage (Kameyama and Gemba, 1991). Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenetic diseases, constituting approximately 5% of all kidney failure diseases (Levy and Feingold, 2000). Genes *PKD1* (The European Polycystic Kidney Disease Consortium, 1994) and *PKD2* (Mochizuki et al., 1996) encode polycystin 1 (PC1) and polycystin 2 (PC2), respectively, that are responsible for ADPKD; yet, the biological function of polycystins remains elusive and controversial (Douguet et al., 2019). Initial studies reported that PC1 and PC2 form a molecular complex configured as a calcium-permeable channel at the plasma membrane of renal tubular cells or neurons (Hanaoka et al., 2000; Delmas et al., 2004). Additionally, these PC1–PC2 complexes located in the primary cilium of kidney cells work as mechano-sensors to the intra-tubular shear stress (Nauli et al., 2003; AbouAlaiwi et al., 2009), but this mechanism has been challenged by subsequent data showing that stimulation of the primary cilium does not induce an increase in intraciliary calcium (Delling et al., 2016). Recently, researchers who focused on localization of the PC complex in MAMs discovered a novel role of these polycystins to regulate mitochondrial function (Padovano et al., 2017). PC1 interacts with the prolyl hydroxylase domain-containing protein (PHD3) to sense local oxygen pressure, and fluctuations in oxygen levels and the PHD3 activity modulate the subcellular localization and the calcium channel activity of the PC complex. PC1-deficient tubular cells had reduced oxygen consumption rate *in vitro*, consistent with a reduction in the quantity of oxidative phosphorylation performed by these cells. Thus, deficiency in the PC1 protein may mimic a relatively low oxygen pressure and lead to mitochondrial dysfunction.

Mitophagy: Mitochondrial Quality Control

Malfunctioning and defective mitochondria are degraded through autophagy known as mitophagy. Impaired mitophagy causes the accumulation of abnormal organelles and severe mitochondrial dysfunction. The PTEN-induced kinase 1 (PINK1)–Parkin pathway is the most widely studied mechanism of mitophagy of neuron (Pickrell and Youle, 2015). Initially, both PINK1 and Parkin were known to be causative mutations in juvenile Parkinson's disease. For example, once mitochondria are damaged and have reduced membrane potential, PINK1 accumulates on the OMM, which recruits the E3 ubiquitin ligase, Parkin, for ubiquitination of PINK1. Then, light-chain-3 (LC3) receptors accumulate and invoke the autophagosome to degrade the dysfunctional mitochondria. In addition, BCL2 Interacting Protein 3 (BNIP3) and NIX in the OMM also regulate mitophagy (Zhang and Ney, 2009). In hypoxic cells, hypoxia-inducible factor-1 (HIF-1) is stabilized and promotes expression of the target genes that eradicates damaged mitochondria (Zhang and Ney, 2008). Furthermore, FUN14 Domain Containing 1

(FUNDC1) in the OMM associates mitophagy with the LC3 receptor through its LC3-interacting region (LIR) domains under hypoxic conditions (Lv et al., 2017; Zhang et al., 2017). Under normal conditions, FUNDC1 is phosphorylated and this moiety blocks binding to LC3.

The pathogenic role of mitophagy in kidney disease still requires further investigation (Bhatia and Choi, 2019; Wang et al., 2020). Tissue damage induced by renal ischemia is prolonged in mice deficient in PINK1, PARK2, or both, representing severe mitochondrial damage and higher ROS production (Tang et al., 2018). The PINK1-Parkin pathway expression is increased in tubular cells stimulated with cisplatin. Interestingly, the silencing of PINK1 or Parkin attenuates mitophagy and promotes cell apoptosis as visualized with immunofluorescence microscopy (Zhao et al., 2017). The protective effect of PINK1/PARK2-dependent mitophagy can also be demonstrated in AKI induced by contrast media (Yang et al., 2018). The BNIP3 expression in cultured renal tubular cells is enhanced by oxygen–glucose deprivation/reperfusion. Finally, BNIP3-deficient mice demonstrate worsened renal ischemic injury due to impaired mitophagy (Tang et al., 2019). These findings suggest that the proper coordination of mitophagy is critical for protection against acute nephrotoxicity, and chronic renal fibrosis is also regulated by the PINK1–Parkin pathway in macrophages (Bhatia et al., 2019).

Apoptosis

Apoptosis is an active and molecular programmed cell death that requires energy to occur. Oxidant stress, abundant cytokines, or hypoxia deteriorate the mitochondrial membrane potential by excreting ROS and cytochrome c from the mitochondria to trigger apoptosis through the p53 and Bcl-2 family proteins (Susnow et al., 2009; Redza-Dutordoir and Averill-Bates, 2016). Cytochrome c binds to the cytoplasmic caspase-9 and forms an aggregate that activates the caspase-9 and inducing apoptosis (Bratton and Salvesen, 2010).

In a damaged kidney, epithelial cells (Shimizu and Yamanaka, 1993) and partially podocytes (Shankland, 2006) are the principal cell types that undergo cell death. The signaling pathway underlying kidney cell apoptosis is not always p53-mediated. Cisplatin can induce Bax-mediated apoptosis in primary-cultured tubular cells isolated from mice deficient in p53 (Jiang et al., 2009), suggesting only a partial effect of p53 inhibition on cisplatin nephrotoxicity. Conversely, apoptosis of the renal cells may also be beneficial during the recovery phase, and assist in fine-tuning the number of renal cells created by balancing an exaggerated proliferative response.

MITOCHONDRIA AND SARCOPENIA IN CKD

Uremic Sarcopenia

Sarcopenia is the progressive reduction of muscle weight and strength, leading to poor physical activity and quality of life, and even increasing the risk of death. Age-related muscle loss and dysfunction were initially defined as sarcopenia, whereas degradation from a chronic inflammatory or malnutritional illness is classified as secondary sarcopenia. CKD is a chronic illness that exhibits sarcopenia symptoms (Moorthi and Avin, 2017). The muscle mass is reduced in those with a greater amount of albuminuria or a lower glomerular filtration rate (GFR) (Foley et al., 2007). Importantly, CKD patients with sarcopenia show higher rates of mortality and longer hospital stays (Sinkeler et al., 2013; Pereira et al., 2015). Epidemiological evidence suggests that multiple lifestyle and clinical factors contribute to the progression of sarcopenia in CKD, including malnutrition, reduced protein intake, insufficient or deficient exercise, chronic inflammation, metabolic acidosis, atherosclerosis, and a lack of natural vitamin D (Stenvinkel and Alvestrand, 2002; Delano and Moldawer, 2006).

Mitochondrial Dysfunction in Skeletal Muscles With Uremia

Several experiments indicate mitochondrial dysfunction in the skeletal muscle of patients with CKD (Gamboa et al., 2016; Sato et al., 2016; Kikuchi et al., 2019; Thome et al., 2019; Xu et al., 2020). Both mitochondrial volume density and mtDNA copy numbers were decreased in skeletal biopsy specimens sampled from kidney failure patients who underwent HD (Gamboa et al., 2016) or PD (Xu et al., 2020) (**Figures 1B**, **2**). A recent human study assessed the phosphocreatine recovery time constant to measure mitochondrial function in the knee extensors using with ^{31}P magnetic resonance spectroscopy (Gamboa et al., 2020). This study demonstrated that the phosphocreatine recovery was extended in pre-dialysis CKD as well as HD patients, compared to healthy control participants (Gamboa et al., 2020). The mitochondrial dysfunction in human skeletal muscle was also associated with poor physical activity performance when evaluated with a 6-min walk test. This result indicates that uremic sarcopenia is already progressing even before CKD advances (Nishi et al., 2020; Ryan, 2020).

Several studies have recognized the impaired role of the PDHC at a molecular level in the mitochondria of skeletal muscle in CKD sarcopenia (Sato et al., 2016; Thome et al., 2019; Xu et al., 2020). The PDHC is essential to energy production under anaerobic condition, as the enzyme converts pyruvate to acetyl-CoA for the TCA cycle. Insufficient activation of PDHC prevents TCA cycle metabolism and reduces ATP production in the mitochondria, resulting in an energy shortage in the skeletal muscle. The activity of the PDHC is regulated by the expression of several kinases and phosphatase via reversible phosphorylation. Inhibition occurs when phosphorylated at Ser232, Ser293, and Ser300 of the PDH E1-α subunit by the PDH kinases PDK-1, PDK-2, PDK-3, and PDK-4, and re-activated by dephosphorylation by the two PDH phosphatases PDP1 and PDP2. In murine C2C12 myocytes, cell cultures exposed to a uremic toxin promote glycolysis with excess antioxidative responses, leading to mitochondrial TCA cycle down-regulation and ATP shortage (Sato et al., 2016). Moreover, PDHC activity and phospho-PDH (S293) are decreased in the skeletal muscle of patients with advanced CKD, whereas PDK4 protein levels are upregulated (Xu et al., 2020). Treatment of CKD in mice

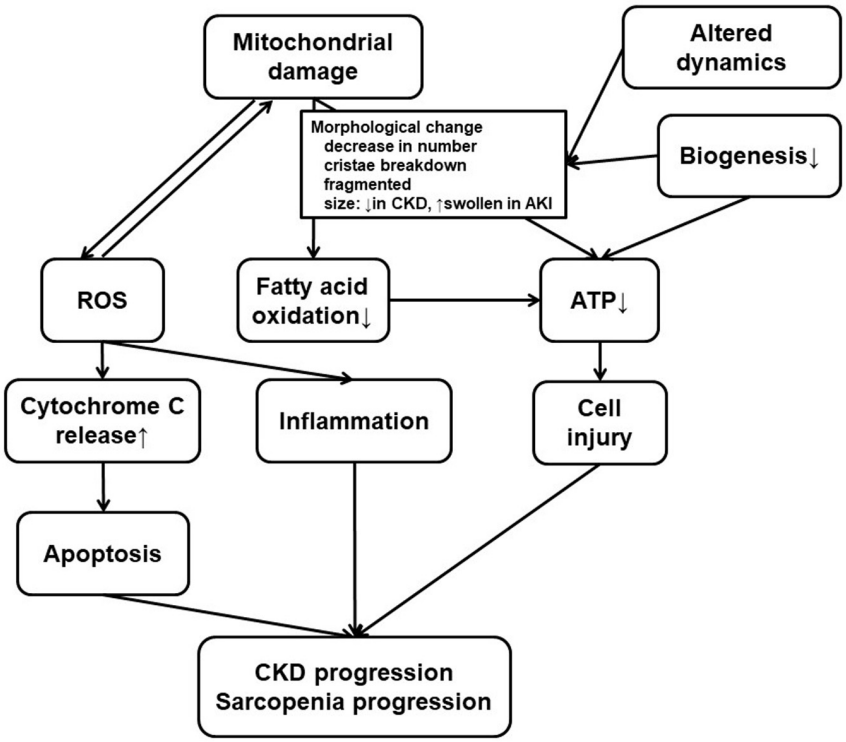


FIGURE 2 | Mitochondrial dysfunction in the kidney and the skeletal muscle. Both kidney failure and sarcopenia are associated with mitochondrial damage, and there are several common findings or processes in the kidney and the skeletal muscle. Mitochondrial damage, usually accompanied with morphological change for altered dynamics and decreased biogenesis, results in ROS accumulation or deficiency in ATP production. ROS production, which can induce mitochondrial damage, promote inflammation or cytochrome C release leading to apoptosis. Low efficiency in ATP production also leads to cell injury. CKD progression or uremic sarcopenia can result from combination of these phenomena. Therapeutics for CKD have various effects on some or all of these processes. At the same time, these processes can still be a novel therapeutic target. CKD, chronic kidney disease; ROS, reactive oxygen species; ATP, adenosine triphosphate.

with dichloroacetate to activate PDHC improved the treadmill running test distance (Tamaki et al., 2014). The uremic condition does not impair the overall activity of mitochondrial enzymes. Isolated mitochondria from murine skeletal muscle had impaired malate and glutamate dehydrogenases, as well as ETC complexes III and IV, by several uremic metabolites (Thome et al., 2019). Treatment with 5-aminolevulinic acid to transport electrons in the mitochondrial ETC also increases the skeletal muscle weight and the mitochondrial amount, thereby improving physical activity (Fujii et al., 2017). Altogether, the reinforcement of muscle mitochondria function serves a potential strategy for eradicating uremic sarcopenia.

DRUGS

Almost no treatments have been approved for slowing or reversing CKD progression, although various treatments for CKD and its complications are currently being trialed. These treatment approaches may potentially have beneficial effects, such as relieving renal fibrosis (a hallmark of CKD) and minimizing AKI in patients, who are predisposed to the development and progression of CKD. This chapter discusses the various therapeutic agents and potentially protective agents for CKD with a focus on the pharmacological effects on the mitochondria in the kidney or skeletal muscle.

Erythropoietin

Erythropoietin (EPO) analogs are a major therapeutic approach to treating anemia from EPO deficiency in CKD. Erythropoietin replenishment has been reported to slow CKD progression (Gouva et al., 2004), as EPO may provide renoprotection from some factors related to the mitochondria. Erythropoietin ameliorates lipopolysaccharide-induced AKI (Stoyanoff et al., 2014). Renoprotection is promoted via an anti-apoptotic effect from the expression of the EPO receptor, the reduction of the Bax/Bcl-XL ratio, the inhibition of cytochrome-c release into the cytosol, and the decrease of active caspase-3 expression. EPO treatment also reduces renal fibrosis in UUO model rats, and downregulates Drp1 overexpression to reduce mitochondria fission (Zhao et al., 2015).

Patients with CKD develop impaired mitochondrial energetics associated with the disease severity in skeletal muscle (Kestenbaum et al., 2020). CKD mice with muscle atrophy have decreased mitochondrial activity and amount, metabolism related to AMP-activated protein kinase (AMPK) phosphorylation and *Pgc1α* gene expression (Tamaki et al., 2014). The EPO receptor expression can be seen in skeletal

muscle (Lamon and Russell, 2013), and EPO treatment increases the PGC-1α protein and gene expression in combination with exercise (Pin et al., 2015). Elevated EPO signaling leads to the activation of mitochondrial biogenesis and metabolism as indicated by increased AMPK phosphorylation, PGC1α expression, and oxygen consumption rate in both *in vitro* and *in vivo* models (Wang et al., 2013). Thus, EPO treatment may alleviate mitochondrial dysfunction in skeletal muscle in CKD and improve muscle amount and performance.

HIF-PH Inhibitor

Hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitors are another emerging therapeutics for renal anemia, as these inhibitors have already been effective in treating patients with or without dialysis (Chen et al., 2019a,b). HIF-PHs stimulates EPO production in the kidney via the activation of the HIF pathway. The agents exert non-hematopoietic effects, as HIF up-regulates the transcription of more than 100 genes involved in hypoxic adaptation. Moreover, HIF-1α regulates cellular metabolism against oxidative phosphorylation via LDHA, PDK, and COX4-2 upregulation and encourages mitochondrial autophagy through BNIP upregulation, resulting in the optimized efficiency of mitochondrial respiration and ROS production (Semenza et al., 1996; Kim et al., 2006; Papandreou et al., 2006; Fukuda et al., 2007; Zhang et al., 2008; Bellot et al., 2009). Moreover, HIF-1α is crucial in preventing mitochondrial dysfunction and apoptosis under hypoxic conditions. Cobalt chloride salt is a classical HIF-PH inhibitor that protects against cisplatin-induced kidney injury in mice (Tanaka et al., 2005). The emerging HIF-PH inhibitor enarodustat also suppresses mitochondrial respiration in HK-2 tubular cells and changes energy metabolism in early DKD mice (Hasegawa et al., 2020).

HIF-1α plays a part in regulation of skeletal muscle function, since a skeletal muscle-specific HIF-1α knockouts in mice have increased mitochondrial activity with higher citrate synthase activity and oxidative metabolism as well as slight increases in the mitochondrial amount and endurance capacity (Mason et al., 2004). Moreover, PHD1 is also associated with mitochondrial changes in skeletal muscle (Aragones et al., 2008). The silencing of PHD1 in myofiber cells decreases oxidative metabolism and less mitochondrial ultrastructural changes compared to ischemia-exhibiting control myofibers with swollen mitochondria, inner lucency, and fractured cristae (Aragones et al., 2008). HIF-PH inhibitors have also been reported to reduce the levels of ROS with glycolytic metabolic shift and increase cell viability in renal proximal tubule cells (Ito et al., 2020) and neuronal cells with glutamate-induced oxytosis (Neitemeier et al., 2016). HIF-PH inhibitor MK-8617 ameliorates myopathy in 5/6 nephrectomy CKD mice and corrects abnormalities in the mitochondrial number and size in skeletal muscle (Qian et al., 2019). Future HIF-PH inhibitors as novel HIF stabilizers may relieve uremic sarcopenia, but they require further investigation.

Nrf2 Activator

The progression of CKD leads to oxidative stress and impaired antioxidant capacity that are associated with the impairment of Nrf2 activity (Ruiz et al., 2013). Bardoxolone methyl, an Nrf2-activating triterpenoid, has been reported to improve renal function in DKD in humans (de Zeeuw et al., 2013), although this drug is not yet approved for patient use. Bardoxolone methyl improved the estimated GFR (eGFR) above baseline in CKD/type 2 diabetes patients in a BEAM randomized, placebo-controlled, 52 week trial (Pergola et al., 2011); however, this trial was prematurely terminated due to as a result of an increased rate of cardiovascular events leading to hospitalization or death in the treatment group because of heart failure (de Zeeuw et al., 2013). Thereafter, the efficacy on DKD was evaluated in a TSUBAKI clinical study in Japan that payed careful attention to cardiac events by excluding patients with risk factors for volume overload or prior history of heart failure (Nangaku et al., 2020). Nrf2 activation also relieves renal injury in non-DKD model mice, and the Nrf2-activating triterpenoid CDDO-imidazolide seems to protect the kidney from ischemia-reperfusion injury by decreasing ROS production in mice (Liu et al., 2014). Another Nrf2 activator, dihydro-CDDO-trifluoroethyl amide (dh404), alleviates proteinuria-induced tubular damage by stopping mitochondrial structural changes, such as decreasing size, number, and breakdown of the cristae structure of mitochondria *in vivo* (Nagasu et al., 2019). Moreover, activator dh404 also decreased mitochondrial ROS and the preservation of mitochondrial membrane potential *in vitro* (Nagasu et al., 2019).

Nrf2 is important for maintaining mitochondrial function, muscle mitohormesis, and oxidative stress defense in skeletal muscle (Coleman et al., 2018; Kitaoka et al., 2019). Moreover, Nrf2 is involved in uremic sarcopenia, which has reduced skeletal muscle mitochondrial mass and gene expression related to mitochondrial biogenesis, including Nrf2 (Liu et al., 2019; Watson et al., 2020). Genetic Nrf2 activation in skeletal muscle improves endurance capacity and increased oxygen consumption without altering mtDNA content (Uruno et al., 2016). Treatment with Nrf2-activating compounds also increases running endurance in rodents (Uruno et al., 2016). Altogether, these results indicate that Nrf2 activators could be effective therapeutic against sarcopenia in CKD patients and require further investigation.

SGLT2 Inhibitor

Recent research has focused on sodium–glucose transporter 2 (SGLT2) inhibitors as a beneficial treatment for DKD. The CREDENCE trial assessed the effects of inhibitor canagliflozin on renal conditions in patients with type 2 diabetes and albuminuric CKD (Perkovic et al., 2019). Canagliflozin had favorable results with a 30% risk reduction in the composite outcome of kidney failure (dialysis, transplantation, or a sustained estimated GFR of <15 ml per minute per 1.73 m^2), a doubling of the serum creatinine level, or death from renal or cardiovascular causes, thereby resulting in early cessation of the trial (Perkovic et al., 2019). Similarly, early cessation of the DAPA-CKD trial was also announced by AstraZeneka (Heerspink et al., 2020), whereas their EMPA-KIDNEY trial is still under way[1]. There are various mechanisms of renoprotection exhibited

[1]www.empakidney.org; accessed April 24, 2020

by the SGLT2 inhibitors, including a decrease in blood glucose levels, tubuloglomerular feedback, upregulation of HIF and EPO, and subsequent hematocrit elevation and oxygen supply (Vallon and Thomson, 2020). The inhibitor ipragliflozin protects tubular cells in the high-fat diet-fed mice (Takagi et al., 2018). These mice show no longer abnormal mitochondrial fission associated with increased oxidative stress, lower gene expression of Opa1 and Mfn2, and higher expression of Drp1 (Takagi et al., 2018). Ipragliflozin also reduces ROS and mitochondrial dysfunction in tubular epithelial cells and glomerular podocytes in diabetic *db/db* mice (Kamezaki et al., 2018).

SGLT2 inhibitors have also been reported to have protective effects against muscle atrophy and lowered exercise performance (Hirata et al., 2019). Empaglifrozin improves symptoms for diabetic sarcopenia in hyperglycemic Akita mice, though it is unclear whether there are other factors beyond the anti-diabetic effects and improving muscle mass (Hirata et al., 2019). Empagliflozin also restores lowered exercise capacity in a murine heart failure model (Nambu et al., 2020). The drug increases endurance capacity, but not muscle weight or muscle strength, by restoring mitochondrial fatty acid oxidation in skeletal muscle (Nambu et al., 2020).

AST-120

AST-120 is an agent that inhibits the accumulation of uremic toxins and is often prescribed to CKD patients to slow the progression of renal failure. The efficacy of AST-120 to slow down CKD progression is controversial, as various clinical trials have failed to show renoprotective effects (Schulman et al., 2015; Cha et al., 2016); however, the drug remains a standard method of treatment for CKD patients.

AST-120 may have beneficial effects on muscles since uremic toxins are harmful to them. AST-120 may improve mitochondrial status by reducing the accumulation of indoxyl sulfate, which induces mitochondrial dysfunction and ATP shortage in muscle cells (Sato et al., 2016). As for *in vivo* models, AST-120 administration improves running endurance reduced in subtotal nephrectomy mice, and attenuates harmful changes, such as down-regulated citrate synthase activity, decreased expression of mitochondrial biogenesis genes like *Pgc1α*, and increased superoxide production (Nishikawa et al., 2015). There are no significant reports on the clinical usage of AST-120 against uremic sarcopenia, so further studies are required to indicate clinical efficacy.

Carnitine

Patients with pre-dialysis CKD have higher plasma L-carnitine levels than healthy individuals (Rodriguez-Segade et al., 1986; Guarnieri et al., 1987). Nevertheless, hemodialysis patients show low plasma and muscle L-carnitine levels that correlate with the dialysis vintage (Sakurauchi et al., 1998; Debska et al., 2000; Evans, 2003).

Carnitine deficiency is associated with EPO-resistant anemia, intradialytic hypotension, cardiomyopathy, and skeletal muscle dysfunction (Karpati et al., 1975). Therefore, L-carnitine supplementation is recommended to relieve such problems (Eknoyan et al., 2003). There is no firm conclusion regarding the clinical efficacy of L-carnitine on skeletal muscle (Hurot et al., 2002), although some trials have shown improvement in muscle volume, strength, and maximal oxygen consumption (Ahmad et al., 1990; Siami et al., 1991). In animal models, L-carnitine improved endurance capacity lowered in CKD mice, normalized PGC-1α expression, and reduced a blunt reduction in type I muscle fibers seen in untreated controls (Enoki et al., 2017).

FUTURE DIRECTIONS

Although mitochondrial dysfunction has been involved in various pathologies, including CKD and sarcopenia, clinical impact of this organelle dysfunction in patients with CKD has not been fully explored. This article reviewed essential mitochondrial functions, mitochondrial changes in CKD and sarcopenia conditions, and the effects of emerging therapeutics on the kidney and skeletal muscle. A comprehensive understanding of mitochondrial physiology is critical for understanding the pathogenesis of kidney diseases and muscle wasting. Furthermore, therapeutic strategies against mitochondrial dysfunctions could lead to drastic progress in the treatment and regression of CKD or sarcopenia.

AUTHOR CONTRIBUTIONS

KT wrote the original manuscript. HN and RI revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research 18K08232 (to HN) and 15KT0088, 16K15465, and 18H02727 (to RI); and Kyowa Kirin Co. Ltd. (to RI). The funder Kyowa Kirin Co. Ltd. was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

REFERENCES

AbouAlaiwi, W. A., Takahashi, M., Mell, B. R., Jones, T. J., Ratnam, S., Kolb, R. J., et al. (2009). Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. *Circ. Res.* 104, 860–869. doi: 10.1161/circresaha.108.192765

Ahmad, S., Robertson, H. T., Golper, T. A., Wolfson, M., Kurtin, P., Katz, L. A., et al. (1990). Multicenter trial of L-carnitine in maintenance hemodialysis patients. II. Clinical and biochemical effects. *Kidney Int.* 38, 912–918. doi: 10.1038/ki.1990.290

Allan, V. J., and Schroer, T. A. (1999). Membrane motors. *Curr. Opin. Cell Biol.* 11, 476–482.

Appenzeller-Herzog, C., and Simmen, T. (2016). ER-luminal thiol/selenol-mediated regulation of Ca2+ signalling. *Biochem. Soc. Trans.* 44, 452–459. doi: 10.1042/bst20150233

Aragones, J., Schneider, M., Van Geyte, K., Fraisl, P., Dresselaers, T., Mazzone, M., et al. (2008). Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat. Genet.* 40, 170–180. doi: 10.1038/ng.2007.62

Ayanga, B. A., Badal, S. S., Wang, Y., Galvan, D. L., Chang, B. H., Schumacker, P. T., et al. (2016). Dynamin-Related protein 1 deficiency improves mitochondrial fitness and protects against progression of diabetic nephropathy. *J. Am. Soc. Nephrol.* 27, 2733–2747. doi: 10.1681/asn.2015101096

Balaban, R. S., and Mandel, L. J. (1980). Coupling of aerobic metabolism to active ion transport in the kidney. *J. Physiol.* 304, 331–348. doi: 10.1113/jphysiol.1980.sp013327

Baverel, G., Forissier, M., and Pellet, M. (1980). Lactate and pyruvate metabolism in dog renal outer medulla. Effects of oleate and ketone bodies. *Int. J. Biochem.* 12, 163–168. doi: 10.1016/b978-0-08-025517-0.50037-0

Bellot, G., Garcia-Medina, R., Gounon, P., Chiche, J., Roux, D., Pouyssegur, J., et al. (2009). Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol. Cell. Biol.* 29, 2570–2581. doi: 10.1128/mcb.00166-09

Benner, C., Konovalov, S., Mackintosh, C., Hutt, K. R., Stunnenberg, R., and Garcia-Bassets, I. (2013). Decoding a signature-based model of transcription cofactor recruitment dictated by cardinal cis-regulatory elements in proximal promoter regions. *PLoS Genet.* 9:e1003906. doi: 10.1371/journal.pgen.1003906

Bhatia, D., and Choi, M. E. (2019). The emerging role of mitophagy in kidney diseases. *J. Life Sci.* 1, 13–22. doi: 10.36069/jols/20191203

Bhatia, D., Chung, K. P., Nakahira, K., Patino, E., Rice, M. C., Torres, L. K., et al. (2019). Mitophagy-dependent macrophage reprogramming protects against kidney fibrosis. *JCI Insight* 4:e132826.

Block, K., Gorin, Y., and Abboud, H. E. (2009). Subcellular localization of Nox4 and regulation in diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14385–14390. doi: 10.1073/pnas.0906805106

Bonifacino, J. S., and Glick, B. S. (2004). The mechanisms of vesicle budding and fusion. *Cell* 116, 153–166. doi: 10.1016/s0092-8674(03)01079-1

Brady, P. S., Ramsay, R. R., and Brady, L. J. (1993). Regulation of the long-chain carnitine acyltransferases. *FASEB J.* 7, 1039–1044. doi: 10.1096/fasebj.7.11.8370473

Bratton, S. B., and Salvesen, G. S. (2010). Regulation of the Apaf-1-caspase-9 apoptosome. *J. Cell Sci.* 123(Pt 19), 3209–3214. doi: 10.1242/jcs.073643

Braun, N., Klumpp, D., Hennenlotter, J., Bedke, J., Duranton, C., Bleif, M., et al. (2015). UCP-3 uncoupling protein confers hypoxia resistance to renal epithelial cells and is upregulated in renal cell carcinoma. *Sci. Rep.* 5:13450.

Bremer, J. (1983). Carnitine–metabolism and functions. *Physiol. Rev.* 63, 1420–1480.

Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., and Sheu, S. S. (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.* 287, C817–C833.

Brooks, C., Wei, Q., Cho, S. G., and Dong, Z. (2009). Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. *J. Clin. Invest.* 119, 1275–1285. doi: 10.1172/jci37829

Cha, R. H., Kang, S. W., Park, C. W., Cha, D. R., Na, K. Y., Kim, S. G., et al. (2016). A Randomized, Controlled Trial of Oral Intestinal Sorbent AST-120 on Renal Function Deterioration in Patients with Advanced Renal Dysfunction. *Clin. J. Am. Soc. Nephrol.* 11, 559–567. doi: 10.2215/cjn.12011214

Chen, N., Hao, C., Liu, B. C., Lin, H., Wang, C., Xing, C., et al. (2019a). Roxadustat treatment for anemia in patients undergoing long-term dialysis. *N. Engl. J. Med.* 381, 1011–1022. doi: 10.1056/nejmoa1901713

Chen, N., Hao, C., Peng, X., Lin, H., Yin, A., Hao, L., et al. (2019b). Roxadustat for anemia in patients with kidney disease not receiving dialysis. *N. Engl. J. Med.* 381, 1001–1010.

Chen, Q., Sun, L., and Chen, Z. J. (2016). Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. *Nat. Immunol.* 17, 1142–1149. doi: 10.1038/ni.3558

Coleman, V., Sa-Nguanmoo, P., Koenig, J., Schulz, T. J., Grune, T., Klaus, S., et al. (2018). Partial involvement of Nrf2 in skeletal muscle mitohormesis as an adaptive response to mitochondrial uncoupling. *Sci. Rep.* 8:2446.

Crane, F. L. (2001). Biochemical functions of coenzyme Q10. *J. Am. Coll. Nutr.* 20, 591–598. doi: 10.1080/07315724.2001.10719063

de Brito, O. M., and Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605–610. doi: 10.1038/nature07534

de Zeeuw, D., Akizawa, T., Audhya, P., Bakris, G. L., Chin, M., Christ-Schmidt, H., et al. (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *N. Engl. J. Med.* 369, 2492–2503.

Debska, S., Kawecka, A., Wojnarowski, K., Prajs, J., Malgorzewicz, S., Kunicka, D., et al. (2000). Correlation between plasma carnitine, muscle carnitine and glycogen levels in maintenance hemodialysis patients. *Int. J. Artif. Organs.* 23, 90–96. doi: 10.1177/039139880002300205

Delano, M. J., and Moldawer, L. L. (2006). The origins of cachexia in acute and chronic inflammatory diseases. *Nutr. Clin. Pract.* 21, 68–81. doi: 10.1177/011542650602100168

Delling, M., Indzhykulian, A. A., Liu, X., Li, Y., Xie, T., Corey, D. P., et al. (2016). Primary cilia are not calcium-responsive mechanosensors. *Nature* 531, 656–660. doi: 10.1038/nature17426

Delmas, P., Nauli, S. M., Li, X., Coste, B., Osorio, N., Crest, M., et al. (2004). Gating of the polycystin ion channel signaling complex in neurons and kidney cells. *FASEB J.* 18, 740–742. doi: 10.1096/fj.03-0319fje

Ding, H., Jiang, L., Xu, J., Bai, F., Zhou, Y., Yuan, Q., et al. (2017). Inhibiting aerobic glycolysis suppresses renal interstitial fibroblast activation and renal fibrosis. *Am. J. Physiol. Renal Physiol.* 313, F561–F575.

Diomedi-Camassei, F., Di Giandomenico, S., Santorelli, F. M., Caridi, G., Piemonte, F., Montini, G., et al. (2007). COQ2 nephropathy: a newly described inherited mitochondriopathy with primary renal involvement. *J. Am. Soc. Nephrol.* 18, 2773–2780. doi: 10.1681/asn.2006080833

Dlaskova, A., Spacek, T., Skobisova, E., Santorova, J., and Jezek, P. (2006). Certain aspects of uncoupling due to mitochondrial uncoupling proteins *in vitro* and *in vivo*. *Biochim. Biophys. Acta* 1757, 467–473. doi: 10.1016/j.bbabio.2006.05.005

Douguet, D., Patel, A., and Honore, E. (2019). Structure and function of polycystins: insights into polycystic kidney disease. *Nat. Rev. Nephrol.* 15, 412–422. doi: 10.1038/s41581-019-0143-6

Eknoyan, G., Latos, D. L., and Lindberg, J. (2003). National Kidney Foundation Carnitine Consensus C. Practice recommendations for the use of L-carnitine in dialysis-related carnitine disorder. National Kidney Foundation Carnitine Consensus Conference. *Am. J. Kidney Dis.* 41, 868–876. doi: 10.1016/s0272-6386(03)00110-0

Enoki, Y., Watanabe, H., Arake, R., Fujimura, R., Ishiodori, K., Imafuku, T., et al. (2017). Potential therapeutic interventions for chronic kidney disease-associated sarcopenia via indoxyl sulfate-induced mitochondrial dysfunction. *J. Cachexia Sarcopenia Muscle* 8, 735–747. doi: 10.1002/jcsm.12202

Ertunc, M. E., and Hotamisligil, G. S. (2016). Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J. Lipid Res.* 57, 2099–2114. doi: 10.1194/jlr.r066514

Evans, A. (2003). Dialysis-related carnitine disorder and levocarnitine pharmacology. *Am. J. Kidney Dis.* 41(4 Suppl. 4), S13–S26.

Eveloff, J., Haase, W., and Kinne, R. (1980). Separation of renal medullary cells: isolation of cells from the thick ascending limb of Henle's loop. *J. Cell Biol.* 87(3 Pt 1), 672–681. doi: 10.1083/jcb.87.3.672

Foley, R. N., Wang, C., Ishani, A., Collins, A. J., and Murray, A. M. (2007). Kidney function and sarcopenia in the United States general population: NHANES III. *Am. J. Nephrol.* 27, 279–286. doi: 10.1159/000101827

Fouad, A. A., Al-Sultan, A. I., Refaie, S. M., and Yacoubi, M. T. (2010). Coenzyme Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice. *Toxicology* 274, 49–56. doi: 10.1016/j.tox.2010.05.007

Friederich, M., Fasching, A., Hansell, P., Nordquist, L., and Palm, F. (2008). Diabetes-induced up-regulation of uncoupling protein-2 results in increased mitochondrial uncoupling in kidney proximal tubular cells. *Biochim. Biophys. Acta* 1777, 935–940. doi: 10.1016/j.bbabio.2008.03.030

Fujii, C., Miyashita, K., Mitsuishi, M., Sato, M., Fujii, K., Inoue, H., et al. (2017). Treatment of sarcopenia and glucose intolerance through mitochondrial activation by 5-aminolevulinic acid. *Sci. Rep.* 7:4013.

Fujimoto, M., and Hayashi, T. (2011). New insights into the role of mitochondria-associated endoplasmic reticulum membrane. *Int. Rev. Cell Mol. Biol.* 292, 73–117. doi: 10.1016/b978-0-12-386033-0.00002-5

Fukuda, R., Zhang, H., Kim, J. W., Shimoda, L., Dang, C. V., and Semenza, G. L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* 129, 111–122. doi: 10.1016/j.cell.2007.01.047

Gamboa, J. L., Billings, F. T., Bojanowski, M. T., Gilliam, L. A., Yu, C., Roshanravan, B., et al. (2016). Mitochondrial dysfunction and oxidative stress in patients with chronic kidney disease. *Physiol. Rep.* 4:e12780.

Gamboa, J. L., Roshanravan, B., Towse, T., Keller, C. A., Falck, A. M., Yu, C., et al. (2020). Skeletal muscle mitochondrial dysfunction is present in patients with CKD before Initiation of Maintenance Hemodialysis. *Clin. J. Am. Soc. Nephrol.* 15, 926–936. doi: 10.2215/cjn.10320819

Gouva, C., Nikolopoulos, P., Ioannidis, J. P., and Siamopoulos, K. C. (2004). Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. *Kidney Int.* 66, 753–760. doi: 10.1111/j.1523-1755.2004.00797.x

Gray, L. R., Tompkins, S. C., and Taylor, E. B. (2014). Regulation of pyruvate metabolism and human disease. *Cell. Mol. Life Sci.* 71, 2577–2604. doi: 10.1007/s00018-013-1539-2

Graziewicz, M. A., Longley, M. J., and Copeland, W. C. (2006). DNA polymerase gamma in mitochondrial DNA replication and repair. *Chem. Rev.* 106, 383–405. doi: 10.1021/cr040463d

Guarnieri, G., Toigo, G., Crapesi, L., Situlin, R., Del Bianco, M. A., Corsi, M., et al. (1987). Carnitine metabolism in chronic renal failure. *Kidney Int.* 22, S116–S127.

Gureev, A. P., Shaforostova, E. A., and Popov, V. N. (2019). Regulation of mitochondrial biogenesis as a way for active longevity: interaction between the Nrf2 and PGC-1alpha signaling pathways. *Front. Genet.* 10:435. doi: 10.3389/fgene.2019.00435

Hanaoka, K., Qian, F., Boletta, A., Bhunia, A. K., Piontek, K., Tsiokas, L., et al. (2000). Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* 408, 990–994. doi: 10.1038/35050128

Hasegawa, S., Tanaka, T., Saito, T., Fukui, K., Wakashima, T., Susaki, E. A., et al. (2020). The oral hypoxia-inducible factor prolyl hydroxylase inhibitor enarodustat counteracts alterations in renal energy metabolism in the early stages of diabetic kidney disease. *Kidney Int.* 97, 934–950. doi: 10.1016/j.kint.2019.12.007

Hayashi, T., and Su, T. P. (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* 131, 596–610. doi: 10.1016/j.cell.2007.08.036

Heerspink, H. J. L., Stefansson, B. V., Chertow, G. M., Correa-Rotter, R., Greene, T., Hou, F. F., et al. (2020). Rationale and protocol of the Dapagliflozin And Prevention of Adverse outcomes in Chronic Kidney Disease (DAPA-CKD) randomized controlled trial. *Nephrol. Dial. Transplant.* 35, 274–282.

Hirata, Y., Nomura, K., Senga, Y., Okada, Y., Kobayashi, K., Okamoto, S., et al. (2019). Hyperglycemia induces skeletal muscle atrophy via a WWP1/KLF15 axis. *JCI Insight* 4:e124952.

Hurot, J. M., Cucherat, M., Haugh, M., and Fouque, D. (2002). Effects of L-carnitine supplementation in maintenance hemodialysis patients: a systematic review. *J. Am. Soc. Nephrol.* 13, 708–714.

Inoue, T., Maekawa, H., and Inagi, R. (2019). Organelle crosstalk in the kidney. *Kidney Int.* 95, 1318–1325. doi: 10.1016/j.kint.2018.11.035

Ishimoto, Y., Inagi, R., Yoshihara, D., Kugita, M., Nagao, S., Shimizu, A., et al. (2017). Mitochondrial abnormality facilitates cyst formation in autosomal dominant polycystic kidney disease. *Mol. Cell Biol.* 37:e00337-17.

Ito, M., Tanaka, T., Ishii, T., Wakashima, T., Fukui, K., and Nangaku, M. (2020). Prolyl hydroxylase inhibition protects the kidneys from ischemia via upregulation of glycogen storage. *Kidney Int.* 97, 687–701. doi: 10.1016/j.kint.2019.10.020

Jha, J. C., Gray, S. P., Barit, D., Okabe, J., El-Osta, A., Namikoshi, T., et al. (2014). Genetic targeting or pharmacologic inhibition of NADPH oxidase nox4 provides renoprotection in long-term diabetic nephropathy. *J. Am. Soc. Nephrol.* 25, 1237–1254. doi: 10.1681/asn.2013070810

Jia, P., Wu, X., Pan, T., Xu, S., Hu, J., and Ding, X. (2019). Uncoupling protein 1 inhibits mitochondrial reactive oxygen species generation and alleviates acute kidney injury. *EBioMedicine* 49, 331–340. doi: 10.1016/j.ebiom.2019.10.023

Jiang, L., Qiu, W., Zhou, Y., Wen, P., Fang, L., Cao, H., et al. (2013). A microRNA-30e/mitochondrial uncoupling protein 2 axis mediates TGF-beta1-induced tubular epithelial cell extracellular matrix production and kidney fibrosis. *Kidney Int.* 84, 285–296. doi: 10.1038/ki.2013.80

Jiang, M., Wang, C. Y., Huang, S., Yang, T., and Dong, Z. (2009). Cisplatin-induced apoptosis in p53-deficient renal cells via the intrinsic mitochondrial pathway. *Am. J. Physiol. Renal Physiol.* 296, F983–F993.

Jornayvaz, F. R., and Shulman, G. I. (2010). Regulation of mitochondrial biogenesis. *Essays Biochem.* 47, 69–84.

Kameyama, Y., and Gemba, M. (1991). Cisplatin-induced injury to calcium uptake by mitochondria in glutathione-depleted slices of rat kidney cortex. *Jpn. J. Pharmacol.* 55, 174–176. doi: 10.1254/jjp.55.174

Kamezaki, M., Kusaba, T., Komaki, K., Fushimura, Y., Watanabe, N., Ikeda, K., et al. (2018). Comprehensive renoprotective effects of ipragliflozin on early diabetic nephropathy in mice. *Sci. Rep.* 8:4029.

Karpati, G., Carpenter, S., Engel, A. G., Watters, G., Allen, J., Rothman, S., et al. (1975). The syndrome of systemic carnitine deficiency. Clinical, morphologic, biochemical, and pathophysiologic features. *Neurology* 25, 16–24. doi: 10.1212/wnl.25.1.16

Katsoulieris, E., Mabley, J. G., Samai, M., Sharpe, M. A., Green, I. C., and Chatterjee, P. K. (2010). Lipotoxicity in renal proximal tubular cells: relationship between endoplasmic reticulum stress and oxidative stress pathways. *Free Radic. Biol. Med.* 48, 1654–1662. doi: 10.1016/j.freeradbiomed.2010.03.021

Kestenbaum, B., Gamboa, J., Liu, S., Ali, A. S., Shankland, E., Jue, T., et al. (2020). Impaired skeletal muscle mitochondrial bioenergetics and physical performance in chronic kidney disease. *JCI Insight* 5:e133289.

Kikuchi, H., Sasaki, E., Nomura, N., Mori, T., Minamishima, Y. A., Yoshizaki, Y., et al. (2019). Failure to sense energy depletion may be a novel therapeutic target in chronic kidney disease. *Kidney Int.* 95, 123–137. doi: 10.1016/j.kint.2018.08.030

Kim, J. W., Tchernyshyov, I., Semenza, G. L., and Dang, C. V. (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* 3, 177–185. doi: 10.1016/j.cmet.2006.02.002

Kitaoka, Y., Tamura, Y., Takahashi, K., Takeda, K., Takemasa, T., and Hatta, H. (2019). Effects of Nrf2 deficiency on mitochondrial oxidative stress in aged skeletal muscle. *Physiol. Rep.* 7:e13998. doi: 10.14814/phy2.13998

Klein, K. I., Wang, M. S., Torikai, S., Davidson, W., and Kurokawa, K. (1980). Substrate oxidation by defined single nephron segments of rat kidney. *Int. J. Biochem.* 12, 53–54. doi: 10.1016/0020-711x(80)90041-5

Lamon, S., and Russell, A. P. (2013). The role and regulation of erythropoietin (EPO) and its receptor in skeletal muscle: how much do we really know? *Front. Physiol.* 4:176. doi: 10.3389/fphys.2013.00176

Lan, R., Geng, H., Singha, P. K., Saikumar, P., Bottinger, E. P., Weinberg, J. M., et al. (2016). Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. *J. Am. Soc. Nephrol.* 27, 3356–3367. doi: 10.1681/asn.2015020177

Lee, J. B., and Peterhm. (1969). Effect of oxygen tension on glucose metabolism in rabbit kidney cortex and medulla. *Am. J. Physiol.* 217, 1464–1471. doi: 10.1152/ajplegacy.1969.217.5.1464

Lee, J. B., Vance, V. K., and Cahill, G. F. Jr. (1962). Metabolism of C14-labeled substrates by rabbit kidney cortex and medulla. *Am. J. Physiol.* 203, 27–36. doi: 10.1152/ajplegacy.1962.203.1.27

Levy, M., and Feingold, J. (2000). Estimating prevalence in single-gene kidney diseases progressing to renal failure. *Kidney Int.* 58, 925–943. doi: 10.1046/j.1523-1755.2000.00250.x

Li, T., and Chen, Z. J. (2018). The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J. Exp. Med.* 215, 1287–1299. doi: 10.1084/jem.20180139

Lira, V. A., Benton, C. R., Yan, Z., and Bonen, A. (2010). PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am. J. Physiol. Endocrinol. Metab.* 299, E145–E161.

Liu, C., Gidlund, E. K., Witasp, A., Qureshi, A. R., Soderberg, M., Thorell, A., et al. (2019). Reduced skeletal muscle expression of mitochondrial-derived peptides humanin and MOTS-C and Nrf2 in chronic kidney disease. *Am. J. Physiol. Renal Physiol.* 317, F1122–F1131.

Liu, M., Reddy, N. M., Higbee, E. M., Potteti, H. R., Noel, S., Racusen, L., et al. (2014). The Nrf2 triterpenoid activator, CDDO-imidazolide, protects kidneys from ischemia-reperfusion injury in mice. *Kidney Int.* 85, 134–141. doi: 10.1038/ki.2013.357

Lv, M., Wang, C., Li, F., Peng, J., Wen, B., Gong, Q., et al. (2017). Structural insights into the recognition of phosphorylated FUNDC1 by LC3B in mitophagy. *Protein Cell* 8, 25–38. doi: 10.1007/s13238-016-0328-8

Maekawa, H., Inoue, T., Ouchi, H., Jao, T. M., Inoue, R., Nishi, H., et al. (2019). Mitochondrial Damage Causes Inflammation via cGAS-STING Signaling in Acute Kidney Injury. *Cell Rep.* 29, 1261–1273.e6.

Mason, S. D., Howlett, R. A., Kim, M. J., Olfert, I. M., Hogan, M. C., McNulty, W., et al. (2004). Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. *PLoS Biol.* 2:e288. doi: 10.1371/journal.pbio.0020288

Mochizuki, T., Wu, G., Hayashi, T., Xenophontos, S. L., Veldhuisen, B., Saris, J. J., et al. (1996). PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272, 1339–1342. doi: 10.1126/science.272.5266.1339

Moorthi, R. N., and Avin, K. G. (2017). Clinical relevance of sarcopenia in chronic kidney disease. *Curr. Opin. Nephrol. Hypertens* 26, 219–228. doi: 10.1097/mnh.0000000000000318

Nagasu, H., Sogawa, Y., Kidokoro, K., Itano, S., Yamamoto, T., Satoh, M., et al. (2019). Bardoxolone methyl analog attenuates proteinuria-induced tubular damage by modulating mitochondrial function. *FASEB J.* 33, 12253–12263. doi: 10.1096/fj.201900217r

Nambu, H., Takada, S., Fukushima, A., Matsumoto, J., Kakutani, N., Maekawa, S., et al. (2020). Empagliflozin restores lowered exercise endurance capacity via the activation of skeletal muscle fatty acid oxidation in a murine model of heart failure. *Eur. J. Pharmacol.* 866:172810. doi: 10.1016/j.ejphar.2019.172810

Nangaku, M., Kanda, H., Takama, H., Ichikawa, T., Hase, H., and Akizawa, T. (2020). Randomized clinical trial on the effect of bardoxolone methyl on GFR in diabetic kidney disease patients (TSUBAKI Study). *Kidney Int. Rep.* 5, 879–890. doi: 10.1016/j.ekir.2020.03.030

Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* 33, 129–137. doi: 10.1038/ng1076

Neitemeier, S., Dolga, A. M., Honrath, B., Karuppagounder, S. S., Alim, I., Ratan, R. R., et al. (2016). Inhibition of HIF-prolyl-4-hydroxylases prevents mitochondrial impairment and cell death in a model of neuronal oxytosis. *Cell Death Dis.* 7:e2214. doi: 10.1038/cddis.2016.107

Nishi, H., Higashihara, T., and Inagi, R. (2019). Lipotoxicity in kidney, heart, and skeletal muscle dysfunction. *Nutrients* 11:1664. doi: 10.3390/nu11071664

Nishi, H., Takemura, K., Higashihara, T., and Inagi, R. (2020). Uremic sarcopenia: clinical evidence and basic experimental approach. *Nutrients* 12:E1814.

Nishikawa, M., Ishimori, N., Takada, S., Saito, A., Kadoguchi, T., Furihata, T., et al. (2015). AST-120 ameliorates lowered exercise capacity and mitochondrial biogenesis in the skeletal muscle from mice with chronic kidney disease via reducing oxidative stress. *Nephrol. Dial. Transplant.* 30, 934–942. doi: 10.1093/ndt/gfv103

Padovano, V., Kuo, I. Y., Stavola, L. K., Aerni, H. R., Flaherty, B. J., Chapin, H. C., et al. (2017). The polycystins are modulated by cellular oxygen-sensing pathways and regulate mitochondrial function. *Mol. Biol. Cell* 28, 261–269. doi: 10.1091/mbc.e16-08-0597

Palou, A., Pico, C., Bonet, M. L., and Oliver, P. (1998). The uncoupling protein, thermogenin. *Int. J. Biochem. Cell Biol.* 30, 7–11. doi: 10.1016/s1357-2725(97)00065-4

Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L., and Denko, N. C. (2006). HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.* 3, 187–197. doi: 10.1016/j.cmet.2006.01.012

Parikh, S. M., Yang, Y., He, L., Tang, C., Zhan, M., and Dong, Z. (2015). Mitochondrial function and disturbances in the septic kidney. *Semin. Nephrol.* 35, 108–119. doi: 10.1016/j.semnephrol.2015.01.011

Pereira, R. A., Cordeiro, A. C., Avesani, C. M., Carrero, J. J., Lindholm, B., Amparo, F. C., et al. (2015). Sarcopenia in chronic kidney disease on conservative therapy: prevalence and association with mortality. *Nephrol. Dial. Transplant.* 30, 1718–1725. doi: 10.1093/ndt/gfv133

Pergola, P. E., Raskin, P., Toto, R. D., Meyer, C. J., Huff, J. W., Grossman, E. B., et al. (2011). Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N. Engl. J. Med.* 365, 327–336.

Perkovic, V., Jardine, M. J., Neal, B., Bompoint, S., Heerspink, H. J. L., Charytan, D. M., et al. (2019). Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N. Engl. J. Med.* 380, 2295–2306.

Perry, H. M., Huang, L., Wilson, R. J., Bajwa, A., Sesaki, H., Yan, Z., et al. (2018). Dynamin-related protein 1 deficiency promotes recovery from AKI. *J. Am. Soc. Nephrol.* 29, 194–206. doi: 10.1681/asn.2017060659

Pickrell, A. M., and Youle, R. J. (2015). The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron* 85, 257–273. doi: 10.1016/j.neuron.2014.12.007

Pin, F., Busquets, S., Toledo, M., Camperi, A., Lopez-Soriano, F. J., Costelli, P., et al. (2015). Combination of exercise training and erythropoietin prevents cancer-induced muscle alterations. *Oncotarget* 6, 43202–43215. doi: 10.18632/oncotarget.6439

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 829–839. doi: 10.1016/s0092-8674(00)81410-5

Qian, F. Y., Li, Z. L., Guo, Y. D., Gao, H. C., Gu, L. H., Le, K., et al. (2019). Hypoxia-inducible factor-prolyl hydroxylase inhibitor ameliorates myopathy in a mouse model of chronic kidney disease. *Am J. Physiol. Renal Physiol.* 317, F1265–F1273.

Raskind, J. Y., and El-Chaar, G. M. (2000). The role of carnitine supplementation during valproic acid therapy. *Ann. Pharmacother.* 34, 630–638. doi: 10.1345/aph.19242

Rector, F. C. Jr. (1983). Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am. J. Physiol.* 244, F461–F471.

Redza-Dutordoir, M., and Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta* 1863, 2977–2992. doi: 10.1016/j.bbamcr.2016.09.012

Ricquier, D., Casteilla, L., and Bouillaud, F. (1991). Molecular studies of the uncoupling protein. *FASEB J.* 5, 2237–2242. doi: 10.1096/fasebj.5.9.1860614

Rodriguez-Segade, S., Alonso de la Pena, C., Paz, M., Novoa, D., Romero, R., Arcocha, V., et al. (1986). Carnitine concentrations in dialysed and undialysed patients with chronic renal insufficiency. *Ann. Clin. Biochem.* 23(Pt 6), 671–675. doi: 10.1177/000456328602300608

Ruiz, S., Pergola, P. E., Zager, R. A., and Vaziri, N. D. (2013). Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int.* 83, 1029–1041. doi: 10.1038/ki.2012.439

Ryan, A. S. (2020). Role of Skeletal Muscle Mitochondrial Dysfunction in CKD. *Clin. J. Am. Soc. Nephrol.* 15, 912–913. doi: 10.2215/cjn.08050520

Sakurauchi, Y., Matsumoto, Y., Shinzato, T., Takai, I., Nakamura, Y., Sato, M., et al. (1998). Effects of L-carnitine supplementation on muscular symptoms in hemodialyzed patients. *Am. J. Kidney Dis.* 32, 258–264. doi: 10.1053/ajkd.1998.v32.pm9708610

Sato, E., Mori, T., Mishima, E., Suzuki, A., Sugawara, S., Kurasawa, N., et al. (2016). Metabolic alterations by indoxyl sulfate in skeletal muscle induce uremic sarcopenia in chronic kidney disease. *Sci. Rep.* 6:36618.

Schrauwen, P., and Hesselink, M. K. (2004). Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes* 53, 1412–1417. doi: 10.2337/diabetes.53.6.1412

Schrauwen, P., Schrauwen-Hinderling, V., Hoeks, J., and Hesselink, M. K. (2010). Mitochondrial dysfunction and lipotoxicity. *Biochim. Biophys. Acta* 1801, 266–271.

Schulman, G., Berl, T., Beck, G. J., Remuzzi, G., Ritz, E., Arita, K., et al. (2015). Randomized placebo-controlled EPPIC Trials of AST-120 in CKD. *J. Am. Soc. Nephrol.* 26, 1732–1746. doi: 10.1681/asn.2014010042

Sedeek, M., Gutsol, A., Montezano, A. C., Burger, D., Nguyen Dinh Cat, A., Kennedy, C. R., et al. (2013). Renoprotective effects of a novel Nox1/4 inhibitor in a mouse model of Type 2 diabetes. *Clin. Sci.* 124, 191–202. doi: 10.1042/cs20120330

Semenza, G. L. (2011). Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim. Biophys. Acta* 1813, 1263–1268. doi: 10.1016/j.bbamcr.2010.08.006

Semenza, G. L., Jiang, B. H., Leung, S. W., Passantino, R., Concordet, J. P., Maire, P., et al. (1996). Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J. Biol. Chem.* 271, 32529–32537. doi: 10.1074/jbc.271.51.32529

Sergi, D., Naumovski, N., Heilbronn, L. K., Abeywardena, M., O'Callaghan, N., Lionetti, L., et al. (2019). Mitochondrial (Dys)function and Insulin Resistance:

from pathophysiological molecular mechanisms to the impact of diet. *Front. Physiol.* 10:532. doi: 10.3389/fphys.2019.00532

Shankland, S. J. (2006). The podocyte's response to injury: role in proteinuria and glomerulosclerosis. *Kidney Int.* 69, 2131–2147. doi: 10.1038/sj.ki.5000410

Shimizu, A., and Yamanaka, N. (1993). Apoptosis and cell desquamation in repair process of ischemic tubular necrosis. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* 64, 171–180. doi: 10.1007/bf02915110

Siami, G., Clinton, M. E., Mrak, R., Griffis, J., and Stone, W. (1991). Evaluation of the effect of intravenous L-carnitine therapy on function, structure and fatty acid metabolism of skeletal muscle in patients receiving chronic hemodialysis. *Nephron* 57, 306–313. doi: 10.1159/000186280

Simon, N., and Hertig, A. (2015). Alteration of fatty acid oxidation in tubular epithelial cells: from acute kidney injury to renal fibrogenesis. *Front. Med.* 2:52. doi: 10.3389/fmed.2015.00052

Sinkeler, S. J., Kwakernaak, A. J., Bakker, S. J., Shahinfar, S., Esmatjes, E., de Zeeuw, D., et al. (2013). Creatinine excretion rate and mortality in type 2 diabetes and nephropathy. *Diabetes Care* 36, 1489–1494. doi: 10.2337/dc12-1545

Son, D., Kojima, I., Inagi, R., Matsumoto, M., Fujita, T., and Nangaku, M. (2008). Chronic hypoxia aggravates renal injury via suppression of Cu/Zn-SOD: a proteomic analysis. *Am. J. Physiol. Renal Physiol.* 294, F62–F72.

Sourris, K. C., Harcourt, B. E., Tang, P. H., Morley, A. L., Huynh, K., Penfold, S. A., et al. (2012). Ubiquinone (coenzyme Q10) prevents renal mitochondrial dysfunction in an experimental model of type 2 diabetes. *Free Radic. Biol. Med.* 52, 716–723. doi: 10.1016/j.freeradbiomed.2011.11.017

Stenvinkel, P., and Alvestrand, A. (2002). Inflammation in end-stage renal disease: sources, consequences, and therapy. *Semin. Dial.* 15, 329–337. doi: 10.1046/j.1525-139x.2002.00083.x

Stewart, V. C., and Heales, S. J. (2003). Nitric oxide-induced mitochondrial dysfunction: implications for neurodegeneration. *Free Radic. Biol. Med.* 34, 287–303. doi: 10.1016/s0891-5849(02)01327-8

Stoyanoff, T. R., Todaro, J. S., Aguirre, M. V., Zimmermann, M. C., and Brandan, N. C. (2014). Amelioration of lipopolysaccharide-induced acute kidney injury by erythropoietin: involvement of mitochondria-regulated apoptosis. *Toxicology* 318, 13–21. doi: 10.1016/j.tox.2014.01.011

Susnow, N., Zeng, L., Margineantu, D., and Hockenbery, D. M. (2009). Bcl-2 family proteins as regulators of oxidative stress. *Semin. Cancer Biol.* 19, 42–49. doi: 10.1016/j.semcancer.2008.12.002

Suzuki, T., Furusato, M., Takasaki, S., and Ishikawa, E. (1975). Giant mitochondria in the epithelial cells of the proximal convoluted tubules of diseased human kidneys. *Lab. Invest.* 33, 578–590.

Takagi, S., Li, J., Takagaki, Y., Kitada, M., Nitta, K., Takasu, T., et al. (2018). Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet. *J Diabetes Investig.* 1025–1032. doi: 10.1111/jdi.12802

Tamaki, M., Miyashita, K., Wakino, S., Mitsuishi, M., Hayashi, K., and Itoh, H. (2014). Chronic kidney disease reduces muscle mitochondria and exercise endurance and its exacerbation by dietary protein through inactivation of pyruvate dehydrogenase. *Kidney Int.* 85, 1330–1339. doi: 10.1038/ki.2013.473

Tanaka, T., Kojima, I., Ohse, T., Inagi, R., Miyata, T., Ingelfinger, J. R., et al. (2005). Hypoxia-inducible factor modulates tubular cell survival in cisplatin nephrotoxicity. *Am. J. Physiol. Renal Physiol.* 289, F1123–F1133.

Tang, C., Han, H., Liu, Z., Liu, Y., Yin, L., Cai, J., et al. (2019). Activation of BNIP3-mediated mitophagy protects against renal ischemia-reperfusion injury. *Cell Death Dis.* 10:677.

Tang, C., Han, H., Yan, M., Zhu, S., Liu, J., Liu, Z., et al. (2018). PINK1-PRKN/PARK2 pathway of mitophagy is activated to protect against renal ischemia-reperfusion injury. *Autophagy* 14, 880–897. doi: 10.1080/15548627.2017.1405880

The European Polycystic Kidney Disease Consortium (1994). The polycystic kidney disease 1 gene encodes a 14kb transcript, and lies within a duplicated region on chromosome 16. *Cell* 77, 881–894. doi: 10.1016/0092-8674(94)90137-6

Thome, T., Salyers, Z. R., Kumar, R. A., Hahn, D., Berru, F. N., Ferreira, L. F., et al. (2019). Uremic metabolites impair skeletal muscle mitochondrial energetics through disruption of the electron transport system and matrix dehydrogenase activity. *Am. J. Physiol. Cell Physiol.* 317, C701–C713.

Toescu, E. C. (2005). Normal brain ageing: models and mechanisms. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 2347–2354. doi: 10.1098/rstb.2005.1771

Uruno, A., Yagishita, Y., Katsuoka, F., Kitajima, Y., Nunomiya, A., Nagatomi, R., et al. (2016). Nrf2-mediated regulation of skeletal muscle glycogen metabolism. *Mol. Cell. Biol.* 36, 1655–1672. doi: 10.1128/mcb.01095-15

Vallon, V., and Thomson, S. C. (2020). The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nat. Rev. Nephrol.* 16, 317–336. doi: 10.1038/s41581-020-0256-y

van Tienen, F. H., Lindsey, P. J., van der Kallen, C. J., and Smeets, H. J. (2010). Prolonged Nrf1 overexpression triggers adipocyte inflammation and insulin resistance. *J. Cell. Biochem.* 111, 1575–1585. doi: 10.1002/jcb.22889

Virbasius, J. V., and Scarpulla, R. C. (1994). Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 91, 1309–1313. doi: 10.1073/pnas.91.4.1309

Wakabayashi, J., Zhang, Z., Wakabayashi, N., Tamura, Y., Fukaya, M., Kensler, T. W., et al. (2009). The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. *J. Cell Biol.* 186, 805–816. doi: 10.1083/jcb.200903065

Wakabayashi, T. (2002). Megamitochondria formation - physiology and pathology. *J. Cell. Mol. Med.* 6, 497–538. doi: 10.1111/j.1582-4934.2002.tb00452.x

Wang, L., Jia, Y., Rogers, H., Suzuki, N., Gassmann, M., Wang, Q., et al. (2013). Erythropoietin contributes to slow oxidative muscle fiber specification via PGC-1alpha and AMPK activation. *Int. J. Biochem. Cell Biol.* 45, 1155–1164. doi: 10.1016/j.biocel.2013.03.007

Wang, Y., Cai, J., Tang, C., and Dong, Z. (2020). Mitophagy in acute kidney injury and kidney repair. *Cells* 9:338. doi: 10.3390/cells9020338

Waterham, H. R., Koster, J., van Roermund, C. W., Mooyer, P. A., Wanders, R. J., and Leonard, J. V. (2007). A lethal defect of mitochondrial and peroxisomal fission. *N. Engl. J. Med.* 356, 1736–1741. doi: 10.1056/nejmoa064436

Watson, E. L., Baker, L. A., Wilkinson, T. J., Gould, D. W., Graham-Brown, M. P. M., Major, R. W., et al. (2020). Reductions in skeletal muscle mitochondrial mass are not restored following exercise training in patients with chronic kidney disease. *FASEB J.* 34, 1755–1767. doi: 10.1096/fj.201901936rr

Wei, Q., Xiao, X., Fogle, P., and Dong, Z. (2014). Changes in metabolic profiles during acute kidney injury and recovery following ischemia/reperfusion. *PLoS One* 9:e106647. doi: 10.1371/journal.pone.0106647

Weidemann, M. J., and Krebs, H. A. (1969). The fuel of respiration of rat kidney cortex. *Biochem. J.* 112, 149–166. doi: 10.1042/bj1120149

Weinberg, J. M. (2006). Lipotoxicity. *Kidney Int.* 70, 1560–1566.

Weisiger, R. A., and Fridovich, I. (1973). Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. *J. Biol. Chem.* 248, 4793–4796.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98, 115–124. doi: 10.1016/s0092-8674(00)80611-x

Xu, C., Kasimumali, A., Guo, X., Lu, R., Xie, K., Zhu, M., et al. (2020). Reduction of mitochondria and up regulation of pyruvate dehydrogenase kinase 4 of skeletal muscle in patients with chronic kidney disease. *Nephrology* 25, 230–238. doi: 10.1111/nep.13606

Yamamoto, T., Takabatake, Y., Takahashi, A., Kimura, T., Namba, T., Matsuda, J., et al. (2017). High-fat diet-induced lysosomal dysfunction and impaired autophagic flux contribute to lipotoxicity in the kidney. *J. Am. Soc. Nephrol.* 28, 1534–1551. doi: 10.1681/asn.2016070731

Yang, M., Li, C., and Sun, L. (2020). Mitochondria-associated membranes (MAMs): a novel therapeutic target for treating metabolic syndrome. *Curr. Med. Chem.* doi: 10.2174/0929867327666200212100644 [Epub ahead of print].

Yang, M., Zhao, L., Gao, P., Zhu, X., Han, Y., Chen, X., et al. (2019). DsbA-L ameliorates high glucose induced tubular damage through maintaining MAM integrity. *EBioMedicine* 43, 607–619. doi: 10.1016/j.ebiom.2019.04.044

Yang, X., Yan, X., Yang, D., Zhou, J., Song, J., and Yang, D. (2018). Rapamycin attenuates mitochondrial injury and renal tubular cell apoptosis in experimental contrast-induced acute kidney injury in rats. *BioSci. Rep.* 38:BSR20180876.

Zhan, M., Usman, I. M., Sun, L., and Kanwar, Y. S. (2015). Disruption of renal tubular mitochondrial quality control by Myo-inositol oxygenase in diabetic kidney disease. *J. Am. Soc. Nephrol.* 26, 1304–1321. doi: 10.1681/asn.2014050457

Zhang, H., Bosch-Marce, M., Shimoda, L. A., Tan, Y. S., Baek, J. H., Wesley, J. B., et al. (2008). Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J. Biol. Chem.* 283, 10892–10903. doi: 10.1074/jbc.m800102200

Zhang, J., and Ney, P. A. (2008). NIX induces mitochondrial autophagy in reticulocytes. *Autophagy* 4, 354–356. doi: 10.4161/auto.5552

Zhang, J., and Ney, P. A. (2009). Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ.* 16, 939–946. doi: 10.1038/cdd.2009.16

Zhang, W., Siraj, S., Zhang, R., and Chen, Q. (2017). Mitophagy receptor FUNDC1 regulates mitochondrial homeostasis and protects the heart from I/R injury. *Autophagy* 13, 1080–1081. doi: 10.1080/15548627.2017.1300224

Zhao, C., Chen, Z., Xu, X., An, X., Duan, S., Huang, Z., et al. (2017). Pink1/Parkin-mediated mitophagy play a protective role in cisplatin induced renal tubular epithelial cells injury. *Exp. Cell Res.* 350, 390–397. doi: 10.1016/j.yexcr.2016.12.015

Zhao, X. F., Liu, Y. H., Han, Z. M., and Xu, Y. U. (2015). Effect of erythropoietin on the expression of dynamin-related protein-1 in rat renal interstitial fibrosis. *Exp. Ther. Med.* 9, 2065–2071. doi: 10.3892/etm.2015.2419

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 04 September 2020
doi: 10.3389/fphys.2020.01079

The Role of Mitochondria in Drug-Induced Kidney Injury

Zhibo Gai[1,2†], Ting Gui[1†], Gerd A. Kullak-Ublick[2,3], Yunlun Li[4,5] and Michele Visentin[2*]*

[1]*Experimental Center, Shandong University of Traditional Chinese Medicine, Jinan, China,* [2]*Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Zurich, Switzerland,* [3]*Mechanistic Safety, CMO & Patient Safety, Global Drug Development, Novartis Pharma, Basel, Switzerland,* [4]*Innovation Research Institute of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China,* [5]*The Third Department of Cardiovascular Diseases, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China*

OPEN ACCESS

Edited by:
Egor Plotnikov, Lomonosov Moscow State University, Russia

Reviewed by:
José Pedraza-Chaverri, National Autonomous University of Mexico, Mexico
Linda A. Gallo, The University of Queensland, Australia
Christian Cortés-Rojo, Michoacana University of San Nicolás de Hidalgo, Mexico

***Correspondence:**
Yunlun Li
yunlun.lee@hotmail.com
Michele Visentin
michele.visentin@usz.ch

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *28 May 2020*
Accepted: *05 August 2020*
Published: *04 September 2020*

Citation:
Gai Z, Gui T, Kullak-Ublick GA, Li Y and Visentin M (2020) The Role of Mitochondria in Drug-Induced Kidney Injury. Front. Physiol. 11:1079. doi: 10.3389/fphys.2020.01079

The kidneys utilize roughly 10% of the body's oxygen supply to produce the energy required for accomplishing their primary function: the regulation of body fluid composition through secreting, filtering, and reabsorbing metabolites and nutrients. To ensure an adequate ATP supply, the kidneys are particularly enriched in mitochondria, having the second highest mitochondrial content and thus oxygen consumption of our body. The bulk of the ATP generated in the kidneys is consumed to move solutes toward (reabsorption) or from (secretion) the peritubular capillaries through the concerted action of an array of ATP-binding cassette (ABC) pumps and transporters. ABC pumps function upon direct ATP hydrolysis. Transporters are driven by the ion electrochemical gradients and the membrane potential generated by the asymmetric transport of ions across the plasma membrane mediated by the ATPase pumps. Some of these transporters, namely the polyspecific organic anion transporters (OATs), the organic anion transporting polypeptides (OATPs), and the organic cation transporters (OCTs) are highly expressed on the proximal tubular cell membranes and happen to also transport drugs whose levels in the proximal tubular cells can rapidly rise, thereby damaging the mitochondria and resulting in cell death and kidney injury. Drug-induced kidney injury (DIKI) is a growing public health concern and a major cause of drug attrition in drug development and post-marketing approval. As part of the article collection "Mitochondria in Renal Health and Disease," here, we provide a critical overview of the main molecular mechanisms underlying the mitochondrial damage caused by drugs inducing nephrotoxicity.

Keywords: antibiotic, anticancer, antiviral, drug-induced kidney injury, mitochondria, oxidative stress, proximal tubular cells

INTRODUCTION

Kidney Disease: Improving Global Outcomes (KDIGO) guidelines define acute kidney injury in patients with normal renal function (serum creatinine of 1.3–1.5 mg/dl) as: (i) a serum creatinine increases by 0.3 mg/dl or more within 48 h, (ii) a serum creatinine increases by 50% or more from the baseline within the previous 7 days, and/or (iii) a urine volume is lower than 0.5 ml/kg/h for 6 h (Palevsky et al., 2013). It has been estimated that 14–26% of all acute kidney injury cases are induced by drugs. Drug-induced kidney injury (DIKI) not

only represents a concerning public health issue but also substantially contributes to the rate of drug attrition in drug development and post-marketing approval (Mehta et al., 2004; Uchino et al., 2005; van der Meer et al., 2010; Moffett and Goldstein, 2011; Hoste et al., 2015). The incidence of DIKI seems to rise when we consider new prescription drugs, likely because (i) some adverse drug reactions can only be observed in large, genetically heterogeneous populations, only reachable several years upon marketing authorization, (ii) recognition of cases of DIKI is particularly challenging for medications not previously associated with kidney damage, because there is no consensus on the definition of DIKI, and (iii) some regulatory agencies have nearly halved the approval review times in the last 20 years, even in the absence of significant advantages for patients in comparison to existing medications (Olson, 2004; van der Meer et al., 2010; Awdishu and Mehta, 2017).

The functional unit of the kidney is the nephron, which is composed of a glomerulus and a tubule. The glomerulus filters the blood, retaining cells and large proteins, producing an ultrafiltrate mainly made of small molecules, from nutrients to ions. The ultrafiltrate enters the tubule, in which highly specialized cells at various segments (proximal tubule, Henle's loop, and distal tubule) contribute to modify the nascent urine by removing substances from the tubular fluid (reabsorption) or adding substances to the tubular fluid (secretion). For a long time, the recognition of the primary site of the renal damage induced by drugs has been complicated because only cases associated with a change in serum creatinine, which is a sign of extended tissue damage to the point of renal dysfunction, were recognized. With the introduction in preclinical and clinical development of a panel of segment-specific markers such as neutrophil gelatinase-associated lipocalin (Ngal; Mishra et al., 2003) and urinary kidney injury molecule-1 (Kim-1; Ichimura et al., 1998; Han et al., 2002), thereby more explicitly addressing the etiology of the renal insult and identifying the injury before the serum creatinine elevates, it could be established that the majority of the DIKI cases have a tubular etiology with primary glomerular injury being relatively uncommon (Mehta et al., 2015). DIKI can be idiosyncratic or intrinsic. Idiosyncratic DIKI is unpredictable and it often stems from a T cell response against the drug triggered by haptens presented by the human leukocyte antigen (HLA) molecules to the T cells (e.g., abacavir). Intrinsic DIKI is the result of a dose-dependent toxic effect of a drug and/or the metabolites thereof, often at the expense of mitochondria, which is followed by cell death and renal dysfunction (Mehta et al., 2015).

Tubular cells are highly enriched in mitochondria to meet the high ATP demand to sustain the activity of the ATPase pumps and the transmembrane electrochemical gradients that drive the secretion and reabsorption of solutes (Soltoff, 1986). Mitochondrial ATP production is strictly oxygen-dependent as the oxidation reaction terminates with the reduction of an oxygen molecule to a water molecule in the final step of the mitochondrial electron transfer. Cytosolic ATP production is instead oxygen-independent and it is exclusively derived from the oxidation of carbohydrates that enter the anaerobic glycolytic pathway. Due to the decreasing corticomedullary oxygen gradient, energy metabolism is quite heterogeneous throughout the tubule. The loop of Henle, which lies in the medullary tract, relies primarily on anaerobic glycolysis, whereas the proximal and distal tubular cells, which resides in the cortex, are enriched in mitochondria and produce ATP primarily from the β-oxidation of fatty acids in the mitochondrial matrix (Clark and Parikh, 2020). Besides being the power plant of the proximal and distal tubular cells, mitochondria are also the hub of reactive oxygen species (ROS) production, which are harmless at low concentrations, but toxic to the mitochondria and the cell at high concentrations (Soltoff, 1986). The proximal segment of the tubule, which is the site of the secretion and the reabsorption of roughly 80% of the glomerular filtrate, is the most enriched in mitochondria (Soltoff, 1986). Another distinctive feature of the proximal tubular cells is the relatively high expression level of a series of polyspecific solute transporters, which can be occasionally hijacked to transport drugs from the peritubular blood into the urine or vice versa (**Table 1**; Fisel et al., 2014). Transport proteins participating in tubular drug secretion and reabsorption are solute carriers (SLCs) and ATP binding cassette (ABC) pumps. The SLCs include organic anion transporters (OATs), organic cation transporters (OCTs), organic anion transporting polypeptides (OATPs), multidrug and toxic compound extrusion (MATE) proteins, and a variety of amino acids and vitamin transporters, such as the folate carriers reduced folate carrier (RFC) and the proton-coupled folate transporter (PCFT). These transporters are passive or secondary active transporters that facilitate the movement of substrates toward or against their electrochemical gradient (Verrey et al., 2009; Zhao et al., 2011; Hagenbuch and Stieger, 2013; Koepsell, 2013; Motohashi and Inui, 2013; Samodelov et al., 2019). The ABC pumps are active transporters with bifunctional features: ATP hydrolysis and transport of the substrate. In the kidneys, the multidrug resistance proteins (MRPs) 2 and 4 and the breast cancer resistance protein (BCRP) appear to be particularly relevant in the elimination of drugs (Leier et al., 2000; Smeets et al., 2004; Huls et al., 2008).

The high expression level of these polyspecific transporters on the membranes of proximal tubular cells and their unique mitochondrial enrichment set the conditions for the "perfect storm," resulting in exposure of mitochondria to high concentration of drugs, massive ATP depletion, intolerable oxidative stress, and proximal tubular cell death. The resulting kidney injury usually manifests as Fanconi's syndrome, a general malabsorption of electrolytes and substances due to proximal tubule malfunctioning that can progress to renal insufficiency (Heidari, 2019). As part of the article collection "Mitochondria in Renal Health and Disease," this article provides an overview of the molecular mechanisms underlying drug-induced mitochondrial damage in DIKI. Sources for this review were obtained through extensive literature searches of publications browsing PubMed. Only papers published in the English language were considered.

MITOCHONDRIAL MEMBRANES

The mitochondrion is made of two membranes, known as the outer mitochondrial membrane and the inner mitochondrial membrane. The outer membrane is smooth and relatively

TABLE 1 | List of drugs associated with acute kidney injury and mitochondrial damage.

Class	Name	Transporter	Target	References
Antibiotic	Colistin	Megalin, PEPT2, and OCTN2	Mitochondrial membrane AMID*	(Teuber and Bader, 1976; Suzuki et al., 2013; Deris et al., 2014; Lu et al., 2016; Visentin et al., 2017)
	Doxycycline	OATs	Mitochondrial ribosome	(Clark-Walker and Linnane, 1966; Babu et al., 2002; Houtkooper et al. 2013; Moullan et al., 2015)
	Gentamicin	Megalin and OCT2	Mitochondrial ribosome Complex II	(Prezant et al., 1993; Moestrup et al., 1995; Qian and Guan, 2009; Gai et al., 2016; O'reilly et al., 2019)
	Vancomycin	OCTs	Complex I	(Sokol, 1991; Arimura et al., 2012; Fujiwara et al., 2012; Sakamoto et al., 2017)
Anticancer	Cisplatin	OCTs	Mitochondrial DNA VDAC	(Yang et al., 2006; Garrido et al., 2008; Filipski et al., 2009; Ciarimboli et al., 2010)
	Gemcitabine	CNTs and ENTs	DNA polimerase γ	(Mackey et al., 1998; Fowler et al., 2008)
	Ifosfamide	OCT2	Complex I	(Nissim et al., 2006; Knouzy et al., 2010; Ciarimboli et al., 2011)
	Methotrexate	FRα, RFC, PCFT, OATs, and OATPs	Mitochondrial TS	(Hoar and Dimnik, 1985; Badagnani et al., 2006; Zhao et al., 2011; Visentin et al., 2012)
	Nitrosourea derivatives	OCTs	Mitochondrial DNA	(Wunderlich et al., 1970; Pettepher et al., 1991; Chen et al., 2001).
Antifungal	Amphotericin B	Unknown	Mitochondrial membrane	(Shekhova et al., 2017)
Antiviral	Zalcitabine, Didanosine, Stavudine, Zidovudine	CNTs and ENTs	DNA polimerase γ	(Birkus et al., 2002; Pinti et al., 2006)
Others	Aristolochic acid	OATs	ANT	(Qi et al., 2007; Bakhiya et al., 2009; Xue et al., 2011)
	Carboxy-atractyloside	Unknown	ANT	(Klingenberg, 2008)

*AMID, apoptosis-inducing factor-homologous mitochondrion associated inducer of death; ANT, adenine nucleotide translocase; CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; FR, folate receptor; OAT, organic anion transporter; OATPs, organic anion transporting polypeptide; OCT, organic cation transporter; PCFT, proton-coupled folate transporter; RFC, reduced folate carrier; TS, thymidylate synthase; VDAC, voltage-dependent anion channel. *Based on phylogenetic analysis.*

permeable to solutes smaller than 5 kDa. It has a low protein density and is considered important for the physical and signaling interaction between the mitochondrion and the other subcellular compartments. The inner mitochondrial membrane is relatively impermeable to solutes and has a high protein:lipid ratio allowed by its deeply convoluted shape (Giacomello et al., 2020). The other distinctive feature of the inner membrane is the presence of cardiolipin, a lipid that is also found in bacterial membranes. Cardiolipin appears to be essential for the mitochondrial respiration and pathological changes in cardiolipin amount or species composition have been shown to alter mitochondrial respiration, boosting the production of reactive oxygen species (Horvath and Daum, 2013; Dudek, 2017).

Experiments in which the polymyxin-resistant *Acholeplasma laidlawii B* bacteria, lacking the cell wall, were fused with vesicles loaded with different lipid species, revealed that cardiolipin and phosphatidylglycerol might represent the polymyxin receptor molecules in the prokaryotic membranes (Teuber and Bader, 1976). Polymyxins are cyclic heptapeptide with an acylated tripeptide side chain, which disrupts the cell membrane integrity of the Gram-negative bacteria (Deris et al., 2014; Yu et al., 2015). Polymyxins are reabsorbed at the level of the proximal tubule by endocytosis and facilitated diffusion. The endocytic uptake seems to involve the glycoprotein megalin (Suzuki et al., 2013). In contrast, the human peptide transporter 2 (PEPT2) and the carnitine/organic cation transporter novel 2 (OCTN2), both highly expressed on the brush border membrane of proximal tubular cells, have been shown to facilitate the transport of polymyxins in overexpressing *in vitro* systems (Horvath and Daum, 2013; Lu et al., 2016; Visentin et al., 2017). Though destabilization of the plasma membrane is considered the primary mechanism of polymyxin toxicity, there are growing evidence that polymyxins induce also mitochondrial damage. The target of polymyxins in mitochondria is not known; however, it might be speculated that the interaction with cardiolipin might trigger mitochondrial membrane depolarization and apoptosis observed in proximal tubular cells (Dai et al., 2013, 2014).

Amphotericin B is an antifungal agent widely administered against invasive fungal infections; however, its clinical application is limited by the frequent onset of nephrotoxicity (Heidari, 2019). The mechanism by which amphotericin B accumulates in the proximal tubular cells is unclear. *In vitro* experiments showed that amphotericin B is a strong inhibitor of the human OCT2 but not its substrate (Trejtnar et al., 2014). Experiments in *Aspergillus fumigatus* showed that amphotericin B binds to the membrane lipids of the mitochondrial membranes, inducing loss of integrity, ROS production, oxidative stress, and lipid peroxidation (Shekhova et al., 2017). Notably, amphotericin B-induced oxidative stress can be markedly reduced by inhibiting the complex I of the electron transport chain, the major site of ROS formation (Mesa-Arango et al., 2014; Shekhova et al., 2017).

MITOCHONDRIAL DNA REPLICATION, TRANSCRIPTION, AND TRANSLATION

Mitochondrial DNA is double stranded like the nuclear DNA, but circular instead of linear. The mitochondrial genome encodes exclusively for two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and the messenger RNA (mRNA) of 13 protein subunits that are assembled with nuclear-encoded proteins to form the four enzymatic complexes constituting the mitochondrial

electron transport chain. Mitochondrial genes are transcribed in a polycistronic manner, whereas nuclear genes are usually transcribed as individual mRNAs. Mitoribosomes, mitochondrial tRNAs, and mRNAs form the translation apparatus; however, the translation process is completely dependent on various nuclear-encoded regulatory proteins (D'souza and Minczuk, 2018). Inhibition of mitochondrial DNA replication and/or protein synthesis, described for multiple classes of drugs associated with acute kidney injury (**Table 1**), results in the reduction of the steady-state level of mitochondrion-encoded proteins, a phenomenon known as mitonuclear protein imbalance, which causes reduced cellular respiration and altered mitochondria dynamics (Houtkooper et al., 2013).

DNA Replication

The replication of the mitochondrial genome relies on the expression and activity of the DNA polymerase γ, which is encoded by the nuclear gene *POLG*. The DNA polymerase γ has two domains: a catalytic one with polymerase activity and an exonuclease one that corrects misincorporations during DNA replication (Kaguni, 2004). Direct inhibition of the DNA polymerase γ activity is considered the mechanism underlying acyclic nucleotide analogue-induced acute kidney injury (Leowattana, 2019). Acyclic nucleotide analogues are widely used to prevent the replication of viruses, such as hepatitis B virus (e.g., lamivudine), hepatitis C virus (e.g., ribavirin), herpes simplex (e.g., acyclovir), and human immunodeficiency virus (HIV; e.g., zidovudine). The uptake and accumulation of acyclic nucleotide analogues are mainly mediated by concentrative nucleoside transporters (CNTs) and equilibrative nucleoside transporters (ENTs) transporters, which are expressed throughout the body and at relatively high levels in the proximal tubular cells. Once inside the cell, acyclic nucleotide analogues must be phosphorylated through three consecutive phosphorylation steps to be able to interact with the viral DNA polymerase. Of crucial importance is the first phosphorylation step, which is the only one ensured by the viral machinery, whereas the other two reactions are mediated by the host kinases. In the triphosphate form, the drugs interact, as competitive inhibitors or alternate substrates, with the dNTPs, and if they are incorporated into the DNA strand, they terminate its extension (De Clercq, 2003). Such interaction is not specific for the viral polymerase. Experiments in different mammalian cells, including human primary renal proximal tubule epithelial cells, with zalcitabine, didanosine, stavudine, and zidovudine suggest that acyclic nucleotide analogues directly interfere with mitochondrial DNA replication catalyzed by the DNA polymerase γ. The ratio between mitochondrial DNA and chromosomal DNA, and the expression of mitochondrial proteins is markedly reduced by the exposure to acyclic nucleotide analogues, leading to mitonuclear protein imbalance. In line with a reduced oxygen-dependent ATP production, the treatment with acyclic nucleotide analogues has been shown to induce the production of lactate *in vitro*, likely as a result of a glycolytic boost (Birkus et al., 2002; Pinti et al., 2006). The inhibition of the DNA polymerase γ by acyclic nucleotide analogues can occur through four different mechanisms: (i) replication is halted, (ii) competitive inhibition with natural nucleotides, (iii) increased error rate in the mitochondrial DNA replication inhibiting the exonucleolytic proofreading function of polymerase γ, and (iv) decreased exonuclease activity (Lewis et al., 2003). It has been found that the extension of the nucleotide moiety through a stable phosphate-carbon bond (phosphonate) results in a markedly reduced mitochondrial and renal toxicity (Verhelst et al., 2002; Izzedine et al., 2005; Fernandez-Fernandez et al., 2011). This chemical modification leads to a profound alteration of the cellular pharmacology and toxicology of acyclic nucleotides: (i) due to their negative charge, the uptake of acyclic nucleotide phosphonates in the kidney cortex is primarily mediated by the organic anion transporter 1 (OAT1) and not by CTNs and ENTs, (ii) acyclic nucleotide phosphonates are direct substrates of the cellular kinases, bypassing the virus-dependent phosphorylation, being effective in the treatment of kinase deficient viruses, (iii) in contrast to the phosphate group (attached through a P-O-C bond), the phosphonate group cannot be cleaved off by cellular hydrolases, facilitating the cellular retention of the drug (Cihlar et al., 1999; Ho et al., 2000; Uwai et al., 2007), and (iv) acyclic nucleotide phosphonates, unlike acyclic nucleotide analogues, are weak inhibitors of the DNA polymerase γ. Though swollen and dysmorphic mitochondria and depletion of mitochondrial DNA have been described in patients who experienced nephrotoxicity upon treatment with acyclic nucleotide phosphonates (Tanji et al., 2001; Saumoy et al., 2004), *in vitro* experiments demonstrated that acyclic nucleotide phosphonates do not interfere with mitochondrial DNA synthesis and do not induce mitochondrial toxicity and renal toxicity in mammalian cells (Birkus et al., 2002). Likely, the mitochondrial toxicity observed in those patients was not related to the exposure to the acyclic nucleotide phosphonates but rather to the co-treatment with acyclic nucleotide analogues and hydroxyurea, both highly nephrotoxic (Tanji et al., 2001; Saumoy et al., 2004; Singh and Xu, 2016). The inhibition of the DNA polymerase γ has been also reported for gemcitabine, a pyrimidine nucleoside analog employed in the treatment of a number of cancers. Though peripheral neuropathy and hematological dysfunction are the most common side effects of this drug, several cases of kidney injury have been reported (Heidari, 2019). Like acyclic nucleotide analogues, gemcitabine is taken up by CNTs and ENTs (Mackey et al., 1998) and accumulates in the cytoplasm and in the mitochondria, inducing swelling of the mitochondria and function alteration (Heidari, 2019). Pre-steady state kinetic experiments showed that gemcitabine directly inhibits the activity of the human DNA polymerase γ (Fowler et al., 2008).

The inhibition of mitochondrial DNA replication can also occur as a consequence of DNA covalent modifications, which are a probable mechanism underlying alkylating agent-induced mitochondrial damage such as platinum and nitrosourea derivatives. Cisplatin, used as first-line treatment of various types of cancer, is a first-generation platinum-based drug and the most nephrotoxic among the platinum derivatives (Miller et al., 2010). The uptake of cisplatin in the proximal tubular cells is primarily mediated by the OCT2, which is highly expressed on the basolateral membrane, thus it is likely to mediate the entry step of cisplatin tubular secretion. After treatment with cisplatin, the double knockout mouse

Oct1/2$^{-/-}$ displayed a milder nephrotoxicity as compared with the wild-type animal. Moreover, patients carrying the nonsynonymous single-nucleotide polymorphism (SNP) in the OCT2 gene *SLC22A2* (rs316019), causing a reduction in the transport activity, have been associated with a reduced risk of cisplatin-induced nephrotoxicity (Filipski et al., 2009; Ciarimboli et al., 2010). There is a large body of evidence that the toxic effects of cisplatin are primarily the result of inter- and intra-strand covalent adduct formation between platinum complexes and DNA. Interestingly, dissociation-enhanced lanthanide fluoroimmunoassay studies performed in Chinese hamster ovary (CHO) cells demonstrated that the rate of cisplatin-DNA adduct incorporation was much higher in mitochondrial DNA than in genomic DNA (Olivero et al., 1995), which leads to inhibition of the DNA amplification by polymerase chain reaction (PCR) and impairment of mitochondrial DNA stability and RNA synthesis (Garrido et al., 2008). Using a panel of cancer cell lines, it has been observed that, unlike the complex I inhibitor rotenone and the uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP), which instantaneously alter cellular oxygen consumption, cisplatin inhibited mitochondrial respiration only after hours of exposure, a timeframe that is consistent with a mitonuclear protein imbalance-dependent effect (Gerschenson et al., 2001; Alborzinia et al., 2011). Noteworthy, it has been shown that there is a positive correlation between the cellular density of mitochondria and cisplatin-induced toxicity (Qian et al., 2005), an observation that provides another explanation for the sensitivity of proximal tubular cells to cisplatin. Damage at the level of the mitochondrial DNA is likely to be also the primary mechanism of nephrotoxicity induced by antineoplastic nitrosourea derivatives (Perry and Weiss, 1982; Narins et al., 1990). After incubation of isolated mitochondria or cell nuclei with radiolabeled methyl-N-nitrosourea, the incorporation into the mitochondrial DNA was 3–7 times that of the nuclear DNA (Wunderlich et al., 1970). Likewise, after intraperitoneal injection in rats of nitrosourea derivatives, the extent of the DNA damage was much higher in the mitochondria than in the nuclei (Wunderlich et al., 1970; Pettepher et al., 1991). The mechanism of accumulation of nitrosourea derivatives in the proximal tubular cells is not fully understood, though association studies have indicated that the expression of organic cation transporters correlated with the cytotoxic effect of nitrosourea derivatives *in vitro* (Noe et al., 1996; Chen et al., 2001). The mitochondrial selectivity of alkylating agents might be explained by a higher accumulation of these drugs in the mitochondria as compared with the nuclei and the lack of histones, which could make the mitochondrial DNA more vulnerable than the genomic DNA to the attack of these alkylating agents (Wunderlich et al., 1970).

Mitochondrial mutations have been observed upon treatment with methotrexate *in vitro*. Methotrexate belongs to the antifolates, the first class of antimetabolites to enter the clinics in the 1950s. Methotrexate inhibits the dihydrofolate reductase, rapidly depleting the cells of tetrahydrofolate derivatives that are key cofactors for the synthesis of thymidylate and purines, the building blocks of DNA and RNA synthesis. The uptake of methotrexate in the proximal tubular cells is mediated by the glycosylphosphatidylinositol (GPI) anchored high-affinity folate receptor α (FRα), by the specific folate transporters RFC and PCFT, and by OATs and OATPs (Badagnani et al., 2006; Zhao et al., 2011; Visentin et al., 2012; Samodelov et al., 2019). The etiology of methotrexate-induced renal dysfunction is believed to be primarily the consequence of the precipitation of the drug in the renal tubules because of its poor solubility at acidic pH (Jacobs et al., 1976; Smeland et al., 1996). Yet, it has been proposed that methotrexate can exert its toxic effect by accumulating in the mitochondrial matrix *via* the mitochondrial folate transporter (MFT; Titus and Moran, 2000). *In vitro* experiments indicated that the mutagenesis induced by methotrexate in non-proliferating cells seems to occur solely at the level of the mitochondrial DNA, sparing the nuclear genome. Because similar results were obtained with 5-fluorodeoxyuridine, the deoxyribonucleoside derivative of 5-fluorouracil, another thymidylate synthase inhibitor widely used in cancer treatment, it is likely that the alteration in the mitochondrial thymidylate pool underlies methotrexate-induced mitochondrial DNA mutations (Hoar and Dimnik, 1985). Notably, though 5-fluorouracil has been shown to cause nephrotoxicity in rats, it is not nephrotoxic in patients at clinically relevant doses (Rashid et al., 2014). The basis for the mitochondrial DNA selectivity of these drugs may be due to the difference in the origin of the thymidylate pool that are incorporated in the mitochondrial DNA and in the nuclear DNA. The *de novo* synthesis of thymidine triphosphate relies on the thymidylate synthase enzyme, which catalyzes the methylation of deoxyuridine monophosphate to thymidine monophosphate. Alternatively, thymidine monophosphate can also synthesize from a salvage pathway catalyzed by the cytosolic thymidine kinase 1. The expression level of thymidylate synthase and thymidine kinase 1 is cell cycle-dependent; thus, it is found in trivial amount in quiescent cells (Ke and Chang, 2004; Le Francois et al., 2007). Mitochondrial thymidine kinase 2 plays a pivotal role in thymidine triphosphate synthesis for mitochondrial DNA replication, which occurs also in non-dividing cells. Because of their differential expression in quiescent cells, such as proximal tubular cells, the inhibition of mitochondrial thymidine kinase 2, rather than thymidylate synthase and thymidine kinase 1, is likely to contribute to methotrexate-induced renal toxicity.

Protein Synthesis

Eukaryotic cells arose from the endosymbiosis of a α-proteobacterium with a host cell with no mitochondria. Mitochondria still harbor remnants of the ancestral bacterial genome and have retained their own transcriptional and translational machinery (Gray et al., 1999). Thus, it is not surprising that mitochondrial protein synthesis is often the target of antibiotics initially developed to inhibit bacterial protein synthesis.

Aminoglycosides are potent inhibitors of the bacterial protein synthesis, whose clinical use is limited by the rapid onset of nephrotoxicity (and ototoxicity). The tropism of aminoglycosides

for the kidneys (and the inner ear) is likely due to the preferential accumulation of this class of antibiotics in these tissues mediated by the low-density lipoprotein receptor-related protein megalin (Moestrup et al., 1995) and the OCT2 (Gai et al., 2016), both highly expressed in these tissues. The relevance of OCT2 in aminoglycoside-induced kidney injury is supported by the clinical observation that male and obese individuals, both characterized by a high expression level of OCT2, carry a higher risk than women or lean individuals of developing toxicity during aminoglycoside therapy (Corcoran et al., 1988; Urakami et al., 2000). Aminoglycosides interfere with bacterial protein synthesis by binding to the internal loop in helix44 of the 16S ribosomal RNA in the 30S subunit of bacterial ribosomes and to the terminal hairpin of helix69 of the bacterial large ribosomal subunit (Borovinskaya et al., 2007), which plays a critical role in ribosomal recycling and translocation (Woodcock et al., 1991; Wang et al., 2012). The aminoglycosides also cause a miscoding of mRNA codons, and, consequently, incorrect amino acids are incorporated into growing peptide chains producing faulty bacterial proteins (Davis, 1987). However, aminoglycosides also bind with lower affinity to mitochondrial ribosomes, exerting a toxic effect also on host cells in the event of high intracellular drug concentrations. By studying the mitochondrial 12S rRNA A1555G mutation, a well-known susceptibility factor in aminoglycoside-induced hearing loss, it was observed that the G1555 variant has higher affinity for aminoglycosides, directly demonstrating that human mitochondrial 12S rRNA is a target of aminoglycosides (Prezant et al., 1993; Qian and Guan, 2009). Moreover, the aminoglycosides exhibit similar binding affinities to the mitochondrial helix69. The interaction enhances the conformational stability of mitochondrial helix69 and increases the structural stability of the rRNA, hindering ribosomal recycling (Hong et al., 2015).

Tetracycline antibiotics are used for the treatment of a broad range of microorganism infections, including those induced by Gram-positive and Gram-negative bacteria. Though they have been primarily associated with liver-induced steatosis, tetracyclines can also induce nephrotoxicity (Lew and French, 1966; Phillips et al., 1974). In the 1960s, several case reports linked the onset of an acquired and usually reversible Fanconi's syndrome with the use of tetracyclines. The authors ascribed the toxic effect to the degradation product epi-anhydrotetracycline, which induced proximal tubular necrosis in rats and dogs (Benitz and Diermeier, 1964; Tapp et al., 1965). Uptake of tetracyclines in the proximal tubular cells is likely mediated by the OAT1, OAT2, OAT3, and OAT4, which are highly expressed on the brush border and the basolateral membranes of the proximal tubular cells (Babu et al., 2002). By exploiting their intrinsic fluorescence, it was demonstrated that tetracyclines rapidly accumulate in the mitochondria (Du Buy and Showacre, 1961). Like aminoglycosides, tetracyclines inhibit bacterial protein synthesis by binding to the ribosomal 30S subunit, preventing the association of aminoacyl-tRNA with the bacterial ribosome (Chopra and Roberts, 2001). Tetracyclines inhibit the translation of proteins encoded by the mitochondrial DNA, but not by the nuclear DNA, leading to mitonuclear protein imbalance (Clark-Walker and Linnane, 1966; Houtkooper et al., 2013; Moullan et al., 2015). Indeed, the gene expression profile of the human bladder cancer cell line RT112 exposed to doxycycline indicated a global repression of the mitochondrial protein synthesis and function (Moullan et al., 2015). Also, mitochondria isolated from the liver of mice treated with doxycycline were characterized by decreased mitochondrial respiration and ATP content, and repression of several mitochondrial genes (Moullan et al., 2015). The extensive experimental evidence on the impact of tetracyclines on mitochondria not only shed light on the molecular mechanisms of tetracycline-induced toxicity but also raised awareness within the scientific community on the use of these antibiotics for inducible gene expression in the Tet-ON/Tet-OFF system. The mitochondrial dysregulation associated with the use of these antibiotics may be a relevant pitfall in the study of mitochondrial-related disorders (Chatzispyrou et al., 2015; Moullan et al., 2015; Luger et al., 2018).

ELECTRON TRANSPORT CHAIN

Reducing equivalents generated from the metabolism of glucose, lipids and amino acids flow into the tricarboxylic acid cycle and the electron transport chain to ultimately produce ATP. The tricarboxylic acid cycle is characterized by eight enzymatic steps, which generate flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (NADH) from FAD and NAD^+. These reduction-oxidation reactions feeds the electron transport chain, a series of multi-subunit proteins, prosthetic groups, and cofactors embedded in the inner mitochondrial membrane. A second series of reduction-oxidation reactions catalyzed by the electron transport chain generates the inward proton gradient required to drive the synthesis of ATP mediated by the F_0F_1-ATP synthase. Five complexes constitute the electron transport chain with complex V being F_0F_1-ATP synthase. Especially, complex I and complex II appear to be the target of a number of widely prescribed drugs (Alberts et al., 2002).

NADH:Ubiquinone Oxidoreductase – Complex I

Complex I oxidizes NADH, transferring two electrons to ubiquinone (Coenzyme Q, CoQ), a lipophilic electron carrier, and four protons into the intermembrane space. Complex I, together with complex II, is the primary source of ROS production in the cell. The most common complex I inhibitor is rotenone though 60 different families of compounds with inhibitory effect at the level of complex I have been identified thus far (Lenaz et al., 2006). Examples of complex I-related DIKI are the anticancer chemotherapeutic ifosfamide and the antibiotic vancomycin.

Ifosfamide is an alkylating agent used in anticancer chemotherapeutic protocols for treating several malignancies, including testicular cancer, soft tissue sarcoma, osteosarcoma, and bladder cancer. The treatment with ifosfamide but not with its structural isomer cyclophosphamide has been associated with tubular dysfunction. The uptake of ifosfamide in proximal tubular cells is mediated primarily by OCT2 and coadministration of cimetidine, another OCT2 substrate, completely prevented

ifosfamide-induced toxicity in isolated mouse proximal tubules. Notably OCT2 does not transport cyclophosphamide (Ciarimboli et al., 2011). The mitochondria of the renal cortex from rats treated with ifosfamide showed a 50% decrease in State 3 respiration with complex I substrates but not with complex II substrates, suggesting that complex I inhibition might underlie ifosfamide-mitochondrial toxicity. Moreover, it has been shown that chloroacetaldehyde, an ifosfamide metabolite, inhibits complex I in rat renal cortical slices as well (Nissim et al., 2006; Knouzy et al., 2010). Co-injection with agmatine, a stimulator of fatty acid β-oxidation, sustained oxidative phosphorylation in the presence of ifosfamide. The authors suggested that agmatine might bypass the block of chloroacetaldehyde at the level of the complex I by feeding the complex II. However, it is worth to mention that agmatine is also a substrate of OCT2, hence the co-administration of agmatine might as well reduce OCT2-mediated uptake of ifosfamide in proximal tubular cells (Nissim et al., 2006; Winter et al., 2011). In patients treated with carboplatin and ifosfamide, who developed Fanconi-like syndrome, decreased expression of the complex III and IV and deletions in mitochondrial DNA were observed (Di Cataldo et al., 1999). However, this finding might be the result of the interaction of carboplatin with mitochondrial and nuclear DNA rather than that of ifosfamide with complex I.

Vancomycin is an amphoteric glycopeptide antibiotic used to treat Gram-positive bacterial infections that do not respond to other antibiotics. Though not fully characterized, vancomycin selectively accumulates in the proximal tubular cells by active tubular secretion from the peritubular blood likely mediated by organic cation transporters (Sokol, 1991; Fujiwara et al., 2012). The only known biological target of vancomycin is the bacterial cell wall. Vancomycin inhibits the biosynthesis of bacterial cell wall peptidoglycan by binding C-terminal acyl-D-alanyl-D-alanine (acyl-d-Ala-d-Ala)-containing residues in peptidoglycan precursors (Reynolds, 1989), a prokaryotic structure. Such selectivity is difficult to reconcile with any off-target effect in the proximal tubular cells, suggesting that, perhaps at very high intracellular concentrations, the vancomycin antibacterial effect might be pleiotropic and most probably less specific. Experiments with LLC-PK1 cells showed that vancomycin binds to and inhibit complex I activity, stimulating the production of superoxide, leading to peroxidation of the mitochondrial phospholipid cardiolipin and mitochondrial membrane depolarization followed by activation of the intrinsic apoptotic pathway. Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), a protonophore that dissipates the proton gradient of the mitochondrial membrane, thereby inhibiting superoxide production and reducing cardiolipin peroxidation, and ameliorates vancomycin mitochondrial toxicity, suggesting that the ROS generated upon complex I inhibition promotes cardiolipin peroxidation followed by mitochondrial membrane depolarization and apoptosis (Orrenius, 2007; Arimura et al., 2012; Hanske et al., 2012; Sakamoto et al., 2017).

There is evidence that polymyxins might inhibit bacterial cellular respiration not only by interacting with the mitochondrial membrane lipids but also by binding to the complex I (Tochikubo et al., 1986; Mogi et al., 2009). The prokaryotic respiratory chain consists of three complexes that move electrons and protons between large protein complexes (Kim and Kim, 2004; Kerscher et al., 2008). Complex I consists of three inner membrane respiratory enzymes: the proton-translocating NADH-quinone (Q) oxidoreductase (NDH-1), the NADH-Q oxidoreductase (NDH-2), and the sodium-translocating NADH-Q oxidoreductase (Yagi et al., 1998). Polymyxins have been shown to inhibit the NDH-2 activity in a non-competitive manner (Deris et al., 2014). Phylogenetic comparisons clustered the human protein apoptosis-inducing factor-homologous mitochondrion associated inducer of death, AMID (AIF-M2) with the prokaryotic NDH-2 family and not in the group containing the canonic AIF proteins, mitochondrial flavoproteins with oxidoreductase activity (Marshall et al., 2005; Elguindy and Nakamaru-Ogiso, 2015; Marreiros et al., 2016).

Succinate-Coenzyme Q Reductase – Complex II

Complex II is the succinate dehydrogenase, an enzyme also involved in the oxidation of succinate to fumarate as part of the tricarboxylic acid cycle. As part of the electron transport chain, succinate dehydrogenase, like complex I, transfers electrons from succinate to ubiquinone (Bezawork-Geleta et al., 2017). Complex II-mediated ROS production is considered key in ischemia/reperfusion injury (Ralph et al., 2011). It has been shown that the antibiotic gentamicin, the most commonly prescribed aminoglycoside, is not only an inhibitor of mitochondrial protein synthesis but can also directly interact with complex II. In cochlear sensory hair cells, gentamicin stimulates state 4 and inhibits state 3u respiratory rates by inhibiting complex II of the electron transport chain, thereby reducing the respiratory control ratio and collapsing the mitochondrial membrane potential as an uncoupler of the electron transport chain. As already known for other uncouplers, gentamicin reduced ROS production in isolated mitochondria (O'reilly et al., 2019). By contrast, intact cells and animals treated with aminoglycosides overproduce ROS (Cuzzocrea et al., 2002; Kalghatgi et al., 2013), perhaps in a mitochondrion-independent manner. In fact, it has been shown that gentamicin can also form ternary complexes with iron and lipid and catalyze ROS formation (Lesniak et al., 2005).

MITOCHONDRIAL PERMEABILITY TRANSITION PORE

The permeability transition represents a sudden increase of the inner mitochondrial membrane permeability to solutes with a molecular weight lower than 1.5 kDa. Short-term openings of the permeability transition pore represent physiological adjustments to regulate Ca^{2+} and ROS homeostasis, providing mitochondria with a fast mechanism to release Ca^{2+} when this reaches a harmful concentration inside the mitochondrial matrix. Conversely, long-term opening of the pore is linked to mitochondrial dysfunction because its occurrence leads to

mitochondrial depolarization, cessation of ATP synthesis, Ca^{2+} release, pyridine nucleotide depletion, and inhibition of respiration. *In vitro*, long-term opening of the pore lead to matrix swelling, which, in turn, causes the mobilization of cytochrome c, the outer mitochondrial membrane rupture, and eventually the release of proapoptotic proteins such as cytochrome c. The precise molecular composition and identity of the mitochondrial permeability transition pore are still controversial. Candidate components of the pore are the adenine nucleotide translocase (ANT), the voltage dependent anion channel (VDAC), the phosphate carrier (PiC), and components of the ATP synthase (Bernardi and Di Lisa, 2015).

It has been suggested that taxane-induced and platinum-induced mitochondrial injury might be associated to a direct interaction with the permeability transition pore. Though there is a large body of evidence that therapeutic and toxic effects of cisplatin on cells is primarily a consequence of inter-strand and intra-strand covalent adduct formation with nucleic acids, it has been shown that platinum can also crosslink with proteins. Experiments in intact cells suggest that cisplatin, perhaps conjugated to glutathione, accumulates in mitochondria, rapidly impairs the oxygen consumption, and induces oxidative stress (Dzamitika et al., 2006; Garrido et al., 2008). Depending on the source, oxidative stress not only can originate from mitochondria but also from other organelles, especially endoplasmic reticulum (ER) and peroxisomes (Kaludercic et al., 2014). Co-localization experiments in Human Kidney-2 (HK-2) cells demonstrated that cisplatin-induced ROS is of mitochondrial origin (Choi et al., 2015). It has been shown that cisplatin can form crosslinks with the VDAC, facilitating mitochondrial membrane permeabilization, release of cytochrome c, and apoptosis (Yang et al., 2006). Short exposure to paclitaxel produces marked loss of renal tubules epithelial lining and damage of the brush border membranes with signs of both oncotic necrosis and apoptosis (Rabah, 2010). Paclitaxel induces an abrupt fall of the mitochondrial membrane potential and a loss of mitochondrial Ca^{2+}. Because cyclosporin A, a de-sensitizer of the mitochondrial permeability transition pore, blocked paclitaxel-induced loss of mitochondrial Ca^{2+}, the authors concluded that paclitaxel induced the opening of the mitochondrial permeability transition pore (Kidd et al., 2002).

The opening of the transition pore has also been associated with nephrotoxicity induced by some natural products very popular in traditional medicine. *Xanthium strumarium*, used in traditional Chinese medicine to treat nasal and sinus congestion, has been linked to several cases of poisoning with renal proximal tubular necrosis features which upon *X. strumarium* ingestion have been reported in the literature (Turgut et al., 2005). The seeds of this plant are enriched in carboxyatractyloside, a well-characterized inhibitor of the ANT. The binding of carboxyatractyloside to the ANT triggers the opening of the mitochondrial transition pore (Obatomi et al., 1998; Klingenberg, 2008). In 1992, a high prevalence of kidney disease in female patients ingesting slimming pills raised attention to the nephrotoxicity of aristolochic acids. Ever since, aristolochic acids are considered a group of toxins that can cause end-stage renal failure associated with urothelial carcinomas (Han et al., 2019). Aristolochic acid is transported into the proximal tubular cells by the OATs (Bakhiya et al., 2009; Xue et al., 2011). Studies in HK-2 cells showed that aristolochic acid exposure caused ATP depletion, mitochondrial membrane depolarization, cytochrome c release, and increase of caspase 3 activity. These toxic effects were attenuated by cyclosporin A, a known "desensitizer" of the opening of the pore (Bernardi, 1996). Experiments in isolated mitochondria showed that aristolochic acid inhibited the activity of the mitochondrial ANT (Qi et al., 2007).

PROTECTIVE STRATEGIES

In principle, there are two approaches to protect the kidneys from drug toxicity. The first approach is reducing the intracellular accumulation of the drug by selectively inhibiting its uptake. A number of works have focused on the pharmacological inhibition of OCT2. It has been shown that the inhibition of OCT2 activity with cimetidine or agmatine, two OCT2 substrates, prevents nephrotoxicity induced by drugs that are OCT2 substrates such as cisplatin and ifosfamide (Nissim et al., 2006; Ciarimboli et al., 2010, 2011). It remains unclear how metformin protects from gentamicin-induced kidney injury. Metformin, a well-known OCT2 substrate, prevents gentamicin-induced nephropathy in rats (Morales et al., 2010; Nasri, 2012). Because at that time a role for OCT2 in gentamicin transport was not considered, the effect of metformin on the renal uptake of gentamicin was not explored. Later on, it was shown that gentamicin is an OCT2 substrate as well, and that gentamicin uptake in primary cultured proximal tubular cells was suppressed by co-incubation with metformin (Gai et al., 2016). To sort out what is the prevalent protective mechanism exerted by metformin, experiments with other mitotoxic drugs that are not substrates of OCT2 and experiments in isolated mitochondria are required. The other protective approach that attracted several research groups is promoting the anti-oxidant activity of the cell, thereby coping better with the high level of oxidative stress subsequent to the mitochondrial injury. There is a plethora of *in vitro* and animal studies showing the nephroprotective effect of different types of antioxidant supplementation in DIKI, yet the subsequent human studies were not just as much practical (Heidari, 2019). Ascorbic acid, a potent reducing agent and radical scavenger, was effective against kidney injury induced by several drugs causing mitochondrial damage, including gentamicin, colistin, and cisplatin (Niki, 1991; May et al., 1995; Antunes et al., 2000; Sharma and Mongan, 2001; Yousef et al., 2012; Moreira et al., 2014). In a small clinical trial, ascorbic acid failed to protect the kidneys of patients taking colistin (Sirijatuphat et al., 2015). Supplementation with N-acetylcysteine, a sulfhydryl-donor with well-known antioxidant properties, hindered the glomerular filtration deterioration in rats treated with amphotericin B (Feldman et al., 2005). Conversely, oral N-acetylcysteine administration to patients under amphotericin B treatment did not prevent the onset of the nephrotoxicity (Karimzadeh et al., 2014).

CONCLUSIONS

Mitochondrial injury is a common event in drug-induced toxicity. The renal liability of some drugs is likely to be the result of the high mitochondrial density in the cytoplasm of tubular cells coupled to the vast array of drug transporters highly expressed on the basolateral and brush border membrane of proximal tubular cells. To improve the safety and thus the therapeutic window of these drugs, the first important step is to study in detail their cellular and molecular pharmacology. It is of particular importance to comprehensively characterize the cellular and subcellular transport and the molecular target(s) of nephrotoxic drugs. Additionally, it is essential to optimize, in humans, dose and treatment schedule of the antioxidant supplementation approaches that have been successfully tested in animals to fill in the shortest time possible, the shortcoming of safe nephroprotective strategies. Information resulting from such targeted studies could be exploited to design pharmacological strategies, which uncouple the uptake of the drug from its toxic effect at the expense of the mitochondria by selective inhibition of the drug uptake into the proximal tubular cells and/or by reducing the oxidative stress burden derived from the mitochondrial damage.

AUTHOR CONTRIBUTIONS

MV contributed to the conceptualization. ZG, TG, and MV contributed to the literature search. ZG, TG, and MV prepared the writing of the original draft preparation. YL and GK-U contributed to the writing-review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the National Natural Science Foundation of China (81774242), the Major Basic Research Projects of Shandong Natural Science Foundation (ZR2018ZC1157), and the Swiss National Science Foundation (310030_175639).

REFERENCES

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). *Molecular biology of the cell. 4th Edn*. New York: Garland Science.

Alborzinia, H., Can, S., Holenya, P., Scholl, C., Lederer, E., Kitanovic, I., et al. (2011). Real-time monitoring of cisplatin-induced cell death. *PLoS One* 6:e19714. doi: 10.1371/journal.pone.0019714

Antunes, L. M., Darin, J. D., and Bianchi, M. D. (2000). Protective effects of vitamin c against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. *Pharmacol. Res.* 41, 405–411. doi: 10.1006/phrs.1999.0600

Arimura, Y., Yano, T., Hirano, M., Sakamoto, Y., Egashira, N., and Oishi, R. (2012). Mitochondrial superoxide production contributes to vancomycin-induced renal tubular cell apoptosis. *Free Radic. Biol. Med.* 52, 1865–1873. doi: 10.1016/j.freeradbiomed.2012.02.038

Awdishu, L., and Mehta, R. L. (2017). The 6R's of drug induced nephrotoxicity. *BMC Nephrol.* 18:124. doi: 10.1186/s12882-017-0536-3

Babu, E., Takeda, M., Narikawa, S., Kobayashi, Y., Yamamoto, T., Cha, S. H., et al. (2002). Human organic anion transporters mediate the transport of tetracycline. *Jpn. J. Pharmacol.* 88, 69–76. doi: 10.1254/jjp.88.69

Badagnani, I., Castro, R. A., Taylor, T. R., Brett, C. M., Huang, C. C., Stryke, D., et al. (2006). Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. *J. Pharmacol. Exp. Ther.* 318, 521–529. doi: 10.1124/jpet.106.104364

Bakhiya, N., Arlt, V. M., Bahn, A., Burckhardt, G., Phillips, D. H., and Glatt, H. (2009). Molecular evidence for an involvement of organic anion transporters (OATs) in aristolochic acid nephropathy. *Toxicology* 264, 74–79. doi: 10.1016/j.tox.2009.07.014

Benitz, K. F., and Diermeier, H. F. (1964). Renal toxicity of tetracycline degradation products. *Proc. Soc. Exp. Biol. Med.* 115, 930–935. doi: 10.3181/00379727-115-29082

Bernardi, P. (1996). The permeability transition pore. control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochim. Biophys. Acta* 1275, 5–9. doi: 10.1016/0005-2728(96)00041-2

Bernardi, P., and Di Lisa, F. (2015). The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. *J. Mol. Cell. Cardiol.* 78, 100–106. doi: 10.1016/j.yjmcc.2014.09.023

Bezawork-Geleta, A., Rohlena, J., Dong, L., Pacak, K., and Neuzil, J. (2017). Mitochondrial complex II: at the crossroads. *Trends Biochem. Sci.* 42, 312–325. doi: 10.1016/j.tibs.2017.01.003

Birkus, G., Hitchcock, M. J., and Cihlar, T. (2002). Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. *Antimicrob. Agents Chemother.* 46, 716–723. doi: 10.1128/AAC.46.3.716-723.2002

Borovinskaya, M. A., Pai, R. D., Zhang, W., Schuwirth, B. S., Holton, J. M., Hirokawa, G., et al. (2007). Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat. Struct. Mol. Biol.* 14, 727–732. doi: 10.1038/nsmb1271

Chatzispyrou, I. A., Held, N. M., Mouchiroud, L., Auwerx, J., and Houtkooper, R. H. (2015). Tetracycline antibiotics impair mitochondrial function and its experimental use confounds research. *Cancer Res.* 75, 4446–4449. doi: 10.1158/0008-5472.CAN-15-1626

Chen, Z. P., Wang, Z. M., Carter, C. A., Alley, M. C., Mohr, G., and Panasci, L. C. (2001). Both extraneuronal monoamine transporter and O(6)-methylguanine-DNA methyltransferase expression influence the antitumor efficacy of 2-chloroethyl-3-sarcosinamide-1-nitrosourea in human tumor xenografts. *J. Pharmacol. Exp. Ther.* 296, 712–715.

Choi, Y. M., Kim, H. K., Shim, W., Anwar, M. A., Kwon, J. W., Kwon, H. K., et al. (2015). Mechanism of cisplatin-induced cytotoxicity is correlated to impaired metabolism due to mitochondrial ROS generation. *PLoS One* 10:e0135083. doi: 10.1371/journal.pone.0135083

Chopra, I., and Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65, 232–260. doi: 10.1128/MMBR.65.2.232-260.2001

Ciarimboli, G., Deuster, D., Knief, A., Sperling, M., Holtkamp, M., Edemir, B., et al. (2010). Organic cation transporter 2 mediates cisplatin-induced oto$^-$ and nephrotoxicity and is a target for protective interventions. *Am. J. Pathol.* 176, 1169–1180. doi: 10.2353/ajpath.2010.090610

Ciarimboli, G., Holle, S. K., Vollenbrocker, B., Hagos, Y., Reuter, S., Burckhardt, G., et al. (2011). New clues for nephrotoxicity induced by ifosfamide: preferential renal uptake via the human organic cation transporter 2. *Mol. Pharm.* 8, 270–279. doi: 10.1021/mp100329u

Cihlar, T., Lin, D. C., Pritchard, J. B., Fuller, M. D., Mendel, D. B., and Sweet, D. H. (1999). The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol. Pharmacol.* 56, 570–580. doi: 10.1124/mol.56.3.570

Clark, A. J., and Parikh, S. M. (2020). Mitochondrial metabolism in acute kidney injury. *Semin. Nephrol.* 40, 101–113. doi: 10.1016/j.semnephrol.2020.01.002

Clark-Walker, G. D., and Linnane, A. W. (1966). In vivo differentiation of yeast cytoplasmic and mitochondrial protein synthesis with antibiotics. *Biochem. Biophys. Res. Commun.* 25, 8–13. doi: 10.1016/0006-291X(66)90631-0

Corcoran, G. B., Salazar, D. E., and Schentag, J. J. (1988). Excessive aminoglycoside nephrotoxicity in obese patients. *Am. J. Med.* 85:279. doi: 10.1016/S0002-9343(88)80370-X

Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Di Paola, R., Britti, D., et al. (2002). A role for superoxide in gentamicin-mediated nephropathy in rats. *Eur. J. Pharmacol.* 450, 67–76. doi: 10.1016/S0014-2999(02)01749-1

Dai, C., Li, J., and Li, J. (2013). New insight in colistin induced neurotoxicity with the mitochondrial dysfunction in mice central nervous tissues. *Exp. Toxicol. Pathol.* 65, 941–948. doi: 10.1016/j.etp.2013.01.008

Dai, C., Li, J., Tang, S., Li, J., and Xiao, X. (2014). Colistin-induced nephrotoxicity in mice involves the mitochondrial, death receptor, and endoplasmic reticulum pathways. *Antimicrob. Agents Chemother.* 58, 4075–4085. doi: 10.1128/AAC.00070-14

Davis, B. D. (1987). Mechanism of bactericidal action of aminoglycosides. *Microbiol. Rev.* 51, 341–350. doi: 10.1128/MMBR.51.3.341-350.1987

De Clercq, E. (2003). Clinical potential of the acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir in treatment of DNA virus and retrovirus infections. *Clin. Microbiol. Rev.* 16, 569–596. doi: 10.1128/cmr.16.4.569-596.2003

Deris, Z. Z., Akter, J., Sivanesan, S., Roberts, K. D., Thompson, P. E., Nation, R. L., et al. (2014). A secondary mode of action of polymyxins against gram-negative bacteria involves the inhibition of NADH-quinone oxidoreductase activity. *J. Antibiot.* 67, 147–151. doi: 10.1038/ja.2013.111

Di Cataldo, A., Palumbo, M., Pittala, D., Renis, M., Schiliro, G., Russo, A., et al. (1999). Deletions in the mitochondrial DNA and decrease in the oxidative phosphorylation activity of children with Fanconi syndrome secondary to antiblastic therapy. *Am. J. Kidney Dis.* 34, 98–106. doi: 10.1016/S0272-6386(99)70114-9

D'souza, A. R., and Minczuk, M. (2018). Mitochondrial transcription and translation: overview. *Essays Biochem.* 62, 309–320. doi: 10.1042/EBC20170102

Du Buy, H. G., and Showacre, J. L. (1961). Selective localization of tetracycline in mitochondria of living cells. *Science* 133, 196–197. doi: 10.1126/science.133.3447.196

Dudek, J. (2017). Role of cardiolipin in mitochondrial signaling pathways. *Front. Cell Dev. Biol.* 5:90. doi: 10.3389/fcell.2017.00090

Dzamitika, S., Salerno, M., Pereira-Maia, E., Le Moyec, L., and Garnier-Suillerot, A. (2006). Preferential energy- and potential-dependent accumulation of cisplatin-gutathione complexes in human cancer cell lines (GLC4 and K562): a likely role of mitochondria. *J. Bioenerg. Biomembr.* 38, 11–21. doi: 10.1007/s10863-006-9001-x

Elguindy, M. M., and Nakamaru-Ogiso, E. (2015). Apoptosis-inducing factor (AIF) and its family member protein, AMID, are rotenone-sensitive NADH:ubiquinone oxidoreductases (NDH-2). *J. Biol. Chem.* 290, 20815–20826. doi: 10.1074/jbc.M115.641498

Feldman, L., Efrati, S., Dishy, V., Katchko, L., Berman, S., Averbukh, M., et al. (2005). N-acetylcysteine ameliorates amphotericin-induced nephropathy in rats. *Nephron Physiol.* 99, 23–27. doi: 10.1159/000081799

Fernandez-Fernandez, B., Montoya-Ferrer, A., Sanz, A. B., Sanchez-Nino, M. D., Izquierdo, M. C., Poveda, J., et al. (2011). Tenofovir nephrotoxicity: 2011 update. *AIDS Res. Treat.* 2011:354908. doi: 10.1155/2011/354908

Filipski, K. K., Mathijssen, R. H., Mikkelsen, T. S., Schinkel, A. H., and Sparreboom, A. (2009). Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin. Pharmacol. Ther.* 86, 396–402. doi: 10.1038/clpt.2009.139

Fisel, P., Renner, O., Nies, A. T., Schwab, M., and Schaeffeler, E. (2014). Solute carrier transporter and drug-related nephrotoxicity: the impact of proximal tubule cell models for preclinical research. *Expert Opin. Drug Metab. Toxicol.* 10, 395–408. doi: 10.1517/17425255.2014.876990

Fowler, J. D., Brown, J. A., Johnson, K. A., and Suo, Z. (2008). Kinetic investigation of the inhibitory effect of gemcitabine on DNA polymerization catalyzed by human mitochondrial DNA polymerase. *J. Biol. Chem.* 283, 15339–15348. doi: 10.1074/jbc.M800310200

Fujiwara, K., Yoshizaki, Y., Shin, M., Miyazaki, T., Saita, T., and Nagata, S. (2012). Immunocytochemistry for vancomycin using a monoclonal antibody that reveals accumulation of the drug in rat kidney and liver. *Antimicrob. Agents Chemother.* 56, 5883–5891. doi: 10.1128/AAC.01267-12

Gai, Z., Visentin, M., Hiller, C., Krajnc, E., Li, T., Zhen, J., et al. (2016). Organic cation transporter 2 overexpression may confer an increased risk of gentamicin-induced nephrotoxicity. *Antimicrob. Agents Chemother.* 60, 5573–5580. doi: 10.1128/AAC.00907-16

Garrido, N., Perez-Martos, A., Faro, M., Lou-Bonafonte, J. M., Fernandez-Silva, P., Lopez-Perez, M. J., et al. (2008). Cisplatin-mediated impairment of mitochondrial DNA metabolism inversely correlates with glutathione levels. *Biochem. J.* 414, 93–102. doi: 10.1042/BJ20071615

Gerschenson, M., Paik, C. Y., Gaukler, E. L., Diwan, B. A., and Poirier, M. C. (2001). Cisplatin exposure induces mitochondrial toxicity in pregnant rats and their fetuses. *Reprod. Toxicol.* 15, 525–531. doi: 10.1016/S0890-6238(01)00156-3

Giacomello, M., Pyakurel, A., Glytsou, C., and Scorrano, L. (2020). The cell biology of mitochondrial membrane dynamics. *Nat. Rev. Mol. Cell Biol.* 21, 204–224. doi: 10.1038/s41580-020-0210-7

Gray, M. W., Burger, G., and Lang, B. F. (1999). Mitochondrial evolution. *Science* 283, 1476–1481. doi: 10.1126/science.283.5407.1476

Hagenbuch, B., and Stieger, B. (2013). The SLCO (former SLC21) superfamily of transporters. *Mol. Asp. Med.* 34, 396–412. doi: 10.1016/j.mam.2012.10.009

Han, W. K., Bailly, V., Abichandani, R., Thadhani, R., and Bonventre, J. V. (2002). Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int.* 62, 237–244. doi: 10.1046/j.1523-1755.2002.00433.x

Han, J., Xian, Z., Zhang, Y., Liu, J., and Liang, A. (2019). Systematic overview of aristolochic acids: nephrotoxicity, carcinogenicity, and underlying mechanisms. *Front. Pharmacol.* 10:648. doi: 10.3389/fphar.2019.00648

Hanske, J., Toffey, J. R., Morenz, A. M., Bonilla, A. J., Schiavoni, K. H., and Pletneva, E. V. (2012). Conformational properties of cardiolipin-bound cytochrome c. *Proc. Natl. Acad. Sci. U. S. A.* 109, 125–130. doi: 10.1073/pnas.1112312108

Heidari, R. (2019). The footprints of mitochondrial impairment and cellular energy crisis in the pathogenesis of xenobiotics-induced nephrotoxicity, serum electrolytes imbalance, and Fanconi's syndrome: a comprehensive review. *Toxicology* 423, 1–31. doi: 10.1016/j.tox.2019.05.002

Ho, E. S., Lin, D. C., Mendel, D. B., and Cihlar, T. (2000). Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J. Am. Soc. Nephrol.* 11, 383–393.

Hoar, D. I., and Dimnik, L. S. (1985). Induction of mitochondrial mutations in human cells by methotrexate. *Basic Life Sci.* 31, 265–282. doi: 10.1007/978-1-4613-2449-2_16

Hong, S., Harris, K. A., Fanning, K. D., Sarachan, K. L., Frohlich, K. M., and Agris, P. F. (2015). Evidence that antibiotics bind to human mitochondrial ribosomal RNA has implications for aminoglycoside toxicity. *J. Biol. Chem.* 290, 19273–19286. doi: 10.1074/jbc.M115.655092

Horvath, S. E., and Daum, G. (2013). Lipids of mitochondria. *Prog. Lipid Res.* 52, 590–614. doi: 10.1016/j.plipres.2013.07.002

Hoste, E. A., Bagshaw, S. M., Bellomo, R., Cely, C. M., Colman, R., Cruz, D. N., et al. (2015). Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med.* 41, 1411–1423. doi: 10.1007/s00134-015-3934-7

Houtkooper, R. H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., et al. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* 497, 451–457. doi: 10.1038/nature12188

Huls, M., Brown, C. D., Windass, A. S., Sayer, R., van den Heuvel, J. J., Heemskerk, S., et al. (2008). The breast cancer resistance protein transporter ABCG2 is expressed in the human kidney proximal tubule apical membrane. *Kidney Int.* 73, 220–225. doi: 10.1038/sj.ki.5002645

Ichimura, T., Bonventre, J. V., Bailly, V., Wei, H., Hession, C. A., Cate, R. L., et al. (1998). Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J. Biol. Chem.* 273, 4135–4142. doi: 10.1074/jbc.273.7.4135

Izzedine, H., Hulot, J. S., Vittecoq, D., Gallant, J. E., Staszewski, S., Launay-Vacher, V., et al. (2005). Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. *Nephrol. Dial. Transplant.* 20, 743–746. doi: 10.1093/ndt/gfh658

Jacobs, S. A., Stoller, R. G., Chabner, B. A., and Johns, D. G. (1976). 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high dose methotrexate. *J. Clin. Invest.* 57, 534–538. doi: 10.1172/JCI108308

Kaguni, L. S. (2004). DNA polymerase gamma, the mitochondrial replicase. *Annu. Rev. Biochem.* 73, 293–320. doi: 10.1146/annurev.biochem.72.121801.161455

Kalghatgi, S., Spina, C. S., Costello, J. C., Liesa, M., Morones-Ramirez, J. R., Slomovic, S., et al. (2013). Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. *Sci. Transl. Med.* 5:192ra185. doi: 10.1126/scitranslmed.3006055

Kaludercic, N., Deshwal, S., and Di Lisa, F. (2014). Reactive oxygen species and redox compartmentalization. *Front. Physiol.* 5:285. doi: 10.3389/fphys.2014.00285

Karimzadeh, I., Khalili, H., Dashti-Khavidaki, S., Sharifian, R., Abdollahi, A., Hasibi, M., et al. (2014). N-acetyl cysteine in prevention of amphotericin-induced electrolytes imbalances: a randomized, double-blinded, placebo-controlled, clinical trial. *Eur. J. Clin. Pharmacol.* 70, 399–408. doi: 10.1007/s00228-014-1642-9

Ke, P. Y., and Chang, Z. F. (2004). Mitotic degradation of human thymidine kinase 1 is dependent on the anaphase-promoting complex/cyclosome-CDH1-mediated pathway. *Mol. Cell. Biol.* 24, 514–526. doi: 10.1128/MCB.24.2.514-526.2004

Kerscher, S., Drose, S., Zickermann, V., and Brandt, U. (2008). The three families of respiratory NADH dehydrogenases. *Results Probl. Cell Differ.* 45, 185–222. doi: 10.1007/400_2007_028

Kidd, J. F., Pilkington, M. F., Schell, M. J., Fogarty, K. E., Skepper, J. N., Taylor, C. W., et al. (2002). Paclitaxel affects cytosolic calcium signals by opening the mitochondrial permeability transition pore. *J. Biol. Chem.* 277, 6504–6510. doi: 10.1074/jbc.M106802200

Kim, M. S., and Kim, Y. J. (2004). Enzymatic properties of the membrane-bound NADH oxidase system in the aerobic respiratory chain of *Bacillus cereus*. *J. Biochem. Mol. Biol.* 37, 753–756. doi: 10.5483/bmbrep.2004.37.6.753

Klingenberg, M. (2008). The ADP and ATP transport in mitochondria and its carrier. *Biochim. Biophys. Acta* 1778, 1978–2021. doi: 10.1016/j.bbamem.2008.04.011

Knouzy, B., Dubourg, L., Baverel, G., and Michoudet, C. (2010). Targets of chloroacetaldehyde-induced nephrotoxicity. *Toxicol. in Vitro* 24, 99–107. doi: 10.1016/j.tiv.2009.08.026

Koepsell, H. (2013). The SLC22 family with transporters of organic cations, anions and zwitterions. *Mol. Asp. Med.* 34, 413–435. doi: 10.1016/j.mam.2012.10.010

Le Francois, B. G., Maroun, J. A., and Birnboim, H. C. (2007). Expression of thymidylate synthase in human cells is an early G(1) event regulated by CDK4 and p16INK4A but not E2F. *Br. J. Cancer* 97, 1242–1250. doi: 10.1038/sj.bjc.6604020

Leier, I., Hummel-Eisenbeiss, J., Cui, Y., and Keppler, D. (2000). ATP-dependent para-aminohippurate transport by apical multidrug resistance protein MRP2. *Kidney Int.* 57, 1636–1642. doi: 10.1046/j.1523-1755.2000.00007.x

Lenaz, G., Fato, R., Genova, M. L., Bergamini, C., Bianchi, C., and Biondi, A. (2006). Mitochondrial complex I: structural and functional aspects. *Biochim. Biophys. Acta* 1757, 1406–1420. doi: 10.1016/j.bbabio.2006.05.007

Leowattana, W. (2019). Antiviral drugs and acute kidney injury (AKI). *Infect. Disord. Drug Targets* 19, 375–382. doi: 10.2174/1871526519666190617154137

Lesniak, W., Pecoraro, V. L., and Schacht, J. (2005). Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. *Chem. Res. Toxicol.* 18, 357–364. doi: 10.1021/tx0496946

Lew, H. T., and French, S. W. (1966). Tetracycline nephrotoxicity and nonoliguric acute renal failure. *Arch. Intern. Med.* 118, 123–128. doi: 10.1001/archinte.1966.00290140027006

Lewis, W., Day, B. J., and Copeland, W. C. (2003). Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat. Rev. Drug Discov.* 2, 812–822. doi: 10.1038/nrd1201

Lu, X., Chan, T., Xu, C., Zhu, L., Zhou, Q. T., Roberts, K. D., et al. (2016). Human oligopeptide transporter 2 (PEPT2) mediates cellular uptake of polymyxins. *J. Antimicrob. Chemother.* 71, 403–412. doi: 10.1093/jac/dkv340

Luger, A. L., Sauer, B., Lorenz, N. I., Engel, A. L., Braun, Y., Voss, M., et al. (2018). Doxycycline impairs mitochondrial function and protects human glioma cells from hypoxia-induced cell death: implications of using tet-inducible systems. *Int. J. Mol. Sci.* 19:1504. doi: 10.3390/ijms19051504

Mackey, J. R., Mani, R. S., Selner, M., Mowles, D., Young, J. D., Belt, J. A., et al. (1998). Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. *Cancer Res.* 58, 4349–4357.

Marreiros, B. C., Sena, F. V., Sousa, F. M., Batista, A. P., and Pereira, M. M. (2016). Type II NADH:quinone oxidoreductase family: phylogenetic distribution, structural diversity and evolutionary divergences. *Environ. Microbiol.* 18, 4697–4709. doi: 10.1111/1462-2920.13352

Marshall, K. R., Gong, M., Wodke, L., Lamb, J. H., Jones, D. J., Farmer, P. B., et al. (2005). The human apoptosis-inducing protein AMID is an oxidoreductase with a modified flavin cofactor and DNA binding activity. *J. Biol. Chem.* 280, 30735–30740. doi: 10.1074/jbc.M414018200

May, J. M., Qu, Z. C., and Whitesell, R. R. (1995). Ascorbate is the major electron donor for a transmembrane oxidoreductase of human erythrocytes. *Biochim. Biophys. Acta* 1238, 127–136. doi: 10.1016/0005-2736(95)00120-r

Mehta, R. L., Awdishu, L., Davenport, A., Murray, P. T., Macedo, E., Cerda, J., et al. (2015). Phenotype standardization for drug-induced kidney disease. *Kidney Int.* 88, 226–234. doi: 10.1038/ki.2015.115

Mehta, R. L., Pascual, M. T., Soroko, S., Savage, B. R., Himmelfarb, J., Ikizler, T. A., et al. (2004). Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int.* 66, 1613–1621. doi: 10.1111/j.1523-1755.2004.00927.x

Mesa-Arango, A. C., Trevijano-Contador, N., Roman, E., Sanchez-Fresneda, R., Casas, C., Herrero, E., et al. (2014). The production of reactive oxygen species is a universal action mechanism of amphotericin B against pathogenic yeasts and contributes to the fungicidal effect of this drug. *Antimicrob. Agents Chemother.* 58, 6627–6638. doi: 10.1128/AAC.03570-14

Miller, R. P., Tadagavadi, R. K., Ramesh, G., and Reeves, W. B. (2010). Mechanisms of cisplatin nephrotoxicity. *Toxins* 2, 2490–2518. doi: 10.3390/toxins2112490

Mishra, J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., et al. (2003). Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J. Am. Soc. Nephrol.* 14, 2534–2543. doi: 10.1097/01.asn.0000088027.54400.c6

Moestrup, S. K., Cui, S., Vorum, H., Bregengard, C., Bjorn, S. E., Norris, K., et al. (1995). Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. *J. Clin. Invest.* 96, 1404–1413. doi: 10.1172/JCI118176

Moffett, B. S., and Goldstein, S. L. (2011). Acute kidney injury and increasing nephrotoxic-medication exposure in noncritically-ill children. *Clin. J. Am. Soc. Nephrol.* 6, 856–863. doi: 10.2215/CJN.08110910

Mogi, T., Murase, Y., Mori, M., Shiomi, K., Omura, S., Paranagama, M. P., et al. (2009). Polymyxin B identified as an inhibitor of alternative NADH dehydrogenase and malate: quinone oxidoreductase from the gram-positive bacterium *Mycobacterium smegmatis*. *J. Biochem.* 146, 491–499. doi: 10.1093/jb/mvp096

Morales, A. I., Detaille, D., Prieto, M., Puente, A., Briones, E., Arevalo, M., et al. (2010). Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. *Kidney Int.* 77, 861–869. doi: 10.1038/ki.2010.11

Moreira, M. A., Nascimento, M. A., Bozzo, T. A., Cintra, A., Da Silva, S. M., Dalboni, M. A., et al. (2014). Ascorbic acid reduces gentamicin-induced nephrotoxicity in rats through the control of reactive oxygen species. *Clin. Nutr.* 33, 296–301. doi: 10.1016/j.clnu.2013.05.005

Motohashi, H., and Inui, K. (2013). Organic cation transporter OCTs (SLC22) and MATEs (SLC47) in the human kidney. *AAPS J.* 15, 581–588. doi: 10.1208/s12248-013-9465-7

Moullan, N., Mouchiroud, L., Wang, X., Ryu, D., Williams, E. G., Mottis, A., et al. (2015). Tetracyclines disturb mitochondrial function across eukaryotic models: a call for caution in biomedical research. *Cell Rep.* 10, 1681–1691. doi: 10.1016/j.celrep.2015.02.034

Narins, R. G., Carley, M., Bloom, E. J., and Harrison, D. S. (1990). The nephrotoxicity of chemotherapeutic agents. *Semin. Nephrol.* 10, 556–564.

Nasri, H. (2012). Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. *J. Res. Med. Sci.* 17, 621–625.

Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr.* 54, 1119S–1124S. doi: 10.1093/ajcn/54.6.1119s

Nissim, I., Horyn, O., Daikhin, Y., Nissim, I., Luhovyy, B., Phillips, P. C., et al. (2006). Ifosfamide-induced nephrotoxicity: mechanism and prevention. *Cancer Res.* 66, 7824–7831. doi: 10.1158/0008-5472.CAN-06-1043

Noe, A. J., Marcantonio, D., Barton, J., Malapetsa, A., and Panasci, L. C. (1996). Characterization of the catecholamine extraneuronal uptake2 carrier in human glioma cell lines SK-MG-1 and SKI-1 in relation to (2-chloroethyl)-3-sarcosinamide-1-nitrosourea (SarCNU) selective cytotoxicity. *Biochem. Pharmacol.* 51, 1639–1648. doi: 10.1016/0006-2952(96)00129-3

Obatomi, D. K., Thanh, N. T., Brant, S., and Bach, P. H. (1998). The toxic mechanism and metabolic effects of atractyloside in precision-cut pig kidney and liver slices. *Arch. Toxicol.* 72, 524–530. doi: 10.1007/s002040050537

Olivero, O. A., Semino, C., Kassim, A., Lopez-Larraza, D. M., and Poirier, M. C. (1995). Preferential binding of cisplatin to mitochondrial DNA of Chinese hamster ovary cells. *Mutat. Res.* 346, 221–230. doi: 10.1016/0165-7992(95)90039-x

Olson, M. K. (2004). Are novel drugs more risky for patients than less novel drugs? *J. Health Econ.* 23, 1135–1158. doi: 10.1016/j.jhealeco.2004.03.007

O'reilly, M., Young, L., Kirkwood, N. K., Richardson, G. P., Kros, C. J., and Moore, A. L. (2019). Gentamicin affects the bioenergetics of isolated mitochondria and collapses the mitochondrial membrane potential in cochlear sensory hair cells. *Front. Cell. Neurosci.* 13:416. doi: 10.3389/fncel.2019.00416

Orrenius, S. (2007). Reactive oxygen species in mitochondria-mediated cell death. *Drug Metab. Rev.* 39, 443–455. doi: 10.1080/03602530701468516

Palevsky, P. M., Liu, K. D., Brophy, P. D., Chawla, L. S., Parikh, C. R., Thakar, C. V., et al. (2013). KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am. J. Kidney Dis.* 61, 649–672. doi: 10.1053/j.ajkd.2013.02.349

Perry, D. J., and Weiss, R. B. (1982). Nephrotoxicity of streptozocin. *Ann. Intern. Med.* 96:122. doi: 10.7326/0003-4819-96-1-122_1

Pettepher, C. C., Ledoux, S. P., Bohr, V. A., and Wilson, G. L. (1991). Repair of alkali-labile sites within the mitochondrial DNA of RINr 38 cells after exposure to the nitrosourea streptozotocin. *J. Biol. Chem.* 266, 3113–3117.

Phillips, M. E., Eastwood, J. B., Curtis, J. R., Gower, P. C., and De Wardener, H. E. (1974). Tetracycline poisoning in renal failure. *Br. Med. J.* 2, 149–151. doi: 10.1136/bmj.2.5911.149

Pinti, M., Salomoni, P., and Cossarizza, A. (2006). Anti-HIV drugs and the mitochondria. *Biochim. Biophys. Acta* 1757, 700–707. doi: 10.1016/j.bbabio.2006.05.001

Prezant, T. R., Agapian, J. V., Bohlman, M. C., Bu, X., Oztas, S., Qiu, W. Q., et al. (1993). Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat. Genet.* 4, 289–294. doi: 10.1038/ng0793-289

Qi, X., Cai, Y., Gong, L., Liu, L., Chen, F., Xiao, Y., et al. (2007). Role of mitochondrial permeability transition in human renal tubular epithelial cell death induced by aristolochic acid. *Toxicol. Appl. Pharmacol.* 222, 105–110. doi: 10.1016/j.taap.2007.03.029

Qian, Y., and Guan, M. X. (2009). Interaction of aminoglycosides with human mitochondrial 12S rRNA carrying the deafness-associated mutation. *Antimicrob. Agents Chemother.* 53, 4612–4618. doi: 10.1128/AAC.00965-08

Qian, W., Nishikawa, M., Haque, A. M., Hirose, M., Mashimo, M., Sato, E., et al. (2005). Mitochondrial density determines the cellular sensitivity to cisplatin-induced cell death. *Am. J. Phys. Cell Physiol.* 289, C1466–C1475. doi: 10.1152/ajpcell.00265.2005

Rabah, S. O. (2010). Acute taxol nephrotoxicity: histological and ultrastructural studies of mice kidney parenchyma. *Saudi J. Biol. Sci.* 17, 105–114. doi: 10.1016/j.sjbs.2010.02.003

Ralph, S. J., Moreno-Sanchez, R., Neuzil, J., and Rodriguez-Enriquez, S. (2011). Inhibitors of succinate: quinone reductase/complex II regulate production of mitochondrial reactive oxygen species and protect normal cells from ischemic damage but induce specific cancer cell death. *Pharm. Res.* 28, 2695–2730. doi: 10.1007/s11095-011-0566-7

Rashid, S., Ali, N., Nafees, S., Hasan, S. K., and Sultana, S. (2014). Mitigation of 5-fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food Chem. Toxicol.* 66, 185–193. doi: 10.1016/j.fct.2014.01.026

Reynolds, P. E. (1989). Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* 8, 943–950. doi: 10.1007/BF01967563

Sakamoto, Y., Yano, T., Hanada, Y., Takeshita, A., Inagaki, F., Masuda, S., et al. (2017). Vancomycin induces reactive oxygen species-dependent apoptosis via mitochondrial cardiolipin peroxidation in renal tubular epithelial cells. *Eur. J. Pharmacol.* 800, 48–56. doi: 10.1016/j.ejphar.2017.02.025

Samodelov, S. L., Gai, Z., Kullak-Ublick, G. A., and Visentin, M. (2019). Renal reabsorption of folates: pharmacological and toxicological snapshots. *Nutrients* 11:2353. doi: 10.3390/nu11102353

Saumoy, M., Vidal, F., Peraire, J., Sauleda, S., Vea, A. M., Vilades, C., et al. (2004). Proximal tubular kidney damage and tenofovir: a role for mitochondrial toxicity? *AIDS* 18, 1741–1742. doi: 10.1097/01.aids.0000131386.38103.37

Sharma, P., and Mongan, P. D. (2001). Ascorbate reduces superoxide production and improves mitochondrial respiratory chain function in human fibroblasts with electron transport chain deficiencies. *Mitochondrion* 1, 191–198. doi: 10.1016/S1567-7249(01)00016-2

Shekhova, E., Kniemeyer, O., and Brakhage, A. A. (2017). Induction of mitochondrial reactive oxygen species production by itraconazole, terbinafine, and amphotericin B as a mode of action against *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* 61, e00978–e00917. doi: 10.1128/AAC.00978-17

Singh, A., and Xu, Y. J. (2016). The cell killing mechanisms of hydroxyurea. *Genes (Basel)* 7:99. doi: 10.3390/genes7110099

Sirijatuphat, R., Limmahakhun, S., Sirivatanauksorn, V., Nation, R. L., Li, J., and Thamlikitkul, V. (2015). Preliminary clinical study of the effect of ascorbic acid on colistin-associated nephrotoxicity. *Antimicrob. Agents Chemother.* 59, 3224–3232. doi: 10.1128/AAC.00280-15

Smeets, P. H., van Aubel, R. A., Wouterse, A. C., van den Heuvel, J. J., and Russel, F. G. (2004). Contribution of multidrug resistance protein 2 (MRP2/ABCC2) to the renal excretion of p-aminohippurate (PAH) and identification of MRP4 (ABCC4) as a novel PAH transporter. *J. Am. Soc. Nephrol.* 15, 2828–2835. doi: 10.1097/01.ASN.0000143473.64430.AC

Smeland, E., Fuskevag, O. M., Nymann, K., Svendesn, J. S., Olsen, R., Lindal, S., et al. (1996). High-dose 7-hydromethotrexate: acute toxicity and lethality in a rat model. *Cancer Chemother. Pharmacol.* 37, 415–422. doi: 10.1007/s002800050406

Sokol, P. P. (1991). Mechanism of vancomycin transport in the kidney: studies in rabbit renal brush border and basolateral membrane vesicles. *J. Pharmacol. Exp. Ther.* 259, 1283–1287.

Soltoff, S. P. (1986). ATP and the regulation of renal cell function. *Annu. Rev. Physiol.* 48, 9–31. doi: 10.1146/annurev.ph.48.030186.000301

Suzuki, T., Yamaguchi, H., Ogura, J., Kobayashi, M., Yamada, T., and Iseki, K. (2013). Megalin contributes to kidney accumulation and nephrotoxicity of colistin. *Antimicrob. Agents Chemother.* 57, 6319–6324. doi: 10.1128/AAC.00254-13

Tanji, N., Tanji, K., Kambham, N., Markowitz, G. S., Bell, A., and D'agati, V. D. (2001). Adefovir nephrotoxicity: possible role of mitochondrial DNA depletion. *Hum. Pathol.* 32, 734–740. doi: 10.1053/hupa.2001.25586

Tapp, E., Carroll, R., and Kovacs, K. (1965). Tetracycline fluorescence in experimental renal lesions. *Arch. Pathol.* 79, 629–634.

Teuber, M., and Bader, J. (1976). Action of polymyxin B on bacterial membranes: phosphatidylglycerol- and cardiolipin-induced susceptibility to polymyxin B in *Acholeplasma laidlawii* B. *Antimicrob. Agents Chemother.* 9, 26–35. doi: 10.1128/AAC.9.1.26

Titus, S. A., and Moran, R. G. (2000). Retrovirally mediated complementation of the glyB phenotype. Cloning of a human gene encoding the carrier for entry of folates into mitochondria. *J. Biol. Chem.* 275, 36811–36817. doi: 10.1074/jbc.M005163200

Tochikubo, K., Yasuda, Y., and Kozuka, S. (1986). Decreased particulate NADH oxidase activity in *Bacillus* subtilis spores after polymyxin B treatment. *J. Gen. Microbiol.* 132, 277–287. doi: 10.1099/00221287-132-2-277

Trejtnar, F., Mandikova, J., Kocincova, J., and Volkova, M. (2014). Renal handling of amphotericin B and amphotericin B-deoxycholate and potential renal drug-drug interactions with selected antivirals. *Antimicrob. Agents Chemother.* 58, 5650–5657. doi: 10.1128/AAC.02829-14

Turgut, M., Alhan, C. C., Gurgoze, M., Kurt, A., Dogan, Y., Tekatli, M., et al. (2005). Carboxyatractyloside poisoning in humans. *Ann. Trop. Paediatr.* 25, 125–134. doi: 10.1179/146532805X45728

Uchino, S., Kellum, J. A., Bellomo, R., Doig, G. S., Morimatsu, H., Morgera, S., et al. (2005). Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 294, 813–818. doi: 10.1001/jama.294.7.813

Urakami, Y., Okuda, M., Saito, H., and Inui, K. (2000). Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett.* 473, 173–176. doi: 10.1016/S0014-5793(00)01525-8

Uwai, Y., Ida, H., Tsuji, Y., Katsura, T., and Inui, K. (2007). Renal transport of adefovir, cidofovir, and tenofovir by SLC22A family members (hOAT1, hOAT3, and hOCT2). *Pharm. Res.* 24, 811–815. doi: 10.1007/s11095-006-9196-x

van der Meer, V., Wielders, H. P., Grootendorst, D. C., De Kanter, J. S., Sijpkens, Y. W., Assendelft, W. J., et al. (2010). Chronic kidney disease in patients with diabetes mellitus type 2 or hypertension in general practice. *Br. J. Gen. Pract.* 60, 884–890. doi: 10.3399/bjgp10X544041

Verhelst, D., Monge, M., Meynard, J. L., Fouqueray, B., Mougenot, B., Girard, P. M., et al. (2002). Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am. J. Kidney Dis.* 40, 1331–1333. doi: 10.1053/ajkd.2002.36924

Verrey, F., Singer, D., Ramadan, T., Vuille-Dit-Bille, R. N., Mariotta, L., and Camargo, S. M. (2009). Kidney amino acid transport. *Pflugers Arch.* 458, 53–60. doi: 10.1007/s00424-009-0638-2

Visentin, M., Gai, Z., Torozi, A., Hiller, C., and Kullak-Ublick, G. A. (2017). Colistin is substrate of the carnitine/organic cation transporter 2 (OCTN2, SLC22A5). *Drug Metab. Dispos.* 45, 1240–1244. doi: 10.1124/dmd.117.077248

Visentin, M., Zhao, R., and Goldman, I. D. (2012). The antifolates. *Hematol. Oncol. Clin. North Am.* 26, 629–648. doi: 10.1016/j.hoc.2012.02.002

Wang, L., Pulk, A., Wasserman, M. R., Feldman, M. B., Altman, R. B., Cate, J. H., et al. (2012). Allosteric control of the ribosome by small-molecule antibiotics. *Nat. Struct. Mol. Biol.* 19, 957–963. doi: 10.1038/nsmb.2360

Winter, T. N., Elmquist, W. F., and Fairbanks, C. A. (2011). OCT2 and MATE1 provide bidirectional agmatine transport. *Mol. Pharm.* 8, 133–142. doi: 10.1021/mp100180a

Woodcock, J., Moazed, D., Cannon, M., Davies, J., and Noller, H. F. (1991). Interaction of antibiotics with A- and P-site-specific bases in 16S ribosomal RNA. *EMBO J.* 10, 3099–3103. doi: 10.1002/j.1460-2075.1991.tb07863.x

Wunderlich, V., Schutt, M., Bottger, M., and Graffi, A. (1970). Preferential alkylation of mitochondrial deoxyribonucleic acid by N-methyl-N-nitrosourea. *Biochem. J.* 118, 99–109. doi: 10.1042/bj1180099

Xue, X., Gong, L. K., Maeda, K., Luan, Y., Qi, X. M., Sugiyama, Y., et al. (2011). Critical role of organic anion transporters 1 and 3 in kidney accumulation and toxicity of aristolochic acid I. *Mol. Pharm.* 8, 2183–2192. doi: 10.1021/mp100418u

Yagi, T., Yano, T., Di Bernardo, S., and Matsuno-Yagi, A. (1998). Procaryotic complex I (NDH-1), an overview. *Biochim. Biophys. Acta* 1364, 125–133. doi: 10.1016/s0005-2728(98)00023-1

Yang, Z., Schumaker, L. M., Egorin, M. J., Zuhowski, E. G., Guo, Z., and Cullen, K. J. (2006). Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. *Clin. Cancer Res.* 12, 5817–5825. doi: 10.1158/1078-0432.CCR-06-1037

Yousef, J. M., Chen, G., Hill, P. A., Nation, R. L., and Li, J. (2012). Ascorbic acid protects against the nephrotoxicity and apoptosis caused by colistin and affects its pharmacokinetics. *J. Antimicrob. Chemother.* 67, 452–459. doi: 10.1093/jac/dkr483

Yu, Z., Qin, W., Lin, J., Fang, S., and Qiu, J. (2015). Antibacterial mechanisms of polymyxin and bacterial resistance. *Biomed. Res. Int.* 2015:679109. doi: 10.1155/2015/679109

Zhao, R., Diop-Bove, N., Visentin, M., and Goldman, I. D. (2011). Mechanisms of membrane transport of folates into cells and across epithelia. *Annu. Rev. Nutr.* 31, 177–201. doi: 10.1146/annurev-nutr-072610-145133

Conflict of Interest: GK-U was employed by the company Novartis Pharma.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 20 October 2020
doi: 10.3389/fphys.2020.566506

Mitochondrial Dysfunction and Kidney Stone Disease

*Sakdithep Chaiyarit and Visith Thongboonkerd**

Medical Proteomics Unit, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Mitochondrion is a pivotal intracellular organelle that plays crucial roles in regulation of energy production, oxidative stress, calcium homeostasis, and apoptosis. Kidney stone disease (nephrolithiasis/urolithiasis), particularly calcium oxalate (CaOx; the most common type), has been shown to be associated with oxidative stress and tissue inflammation/injury. Recent evidence has demonstrated the involvement of mitochondrial dysfunction in CaOx crystal retention and aggregation as well as Randall's plaque formation, all of which are the essential mechanisms for kidney stone formation. This review highlights the important roles of mitochondria in renal cell functions and provides the data obtained from previous investigations of mitochondria related to kidney stone disease. In addition, mechanisms for the involvement of mitochondrial dysfunction in the pathophysiology of kidney stone disease are summarized. Finally, future perspectives on the novel approach to prevent kidney stone formation by mitochondrial preservation are discussed.

Keywords: antioxidant, calcium, calcium oxalate, mitochondria, nephrolithiasis, oxidative stress, reactive oxygen species, urolithiasis

OPEN ACCESS

Edited by:
Egor Plotnikov, Lomonosov Moscow State University, Russia

Reviewed by:
Carlos Palmeira, University of Coimbra, Portugal
Felix Grases, University of the Balearic Islands, Spain
John Andrew Sayer, Newcastle University, United Kingdom

***Correspondence:**
Visith Thongboonkerd
vthongbo@yahoo.com; thongboonkerd@dr.com

Specialty section:
This article was submitted to Mitochondrial Research, a section of the journal Frontiers in Physiology

Received: *28 May 2020*
Accepted: *16 September 2020*
Published: *20 October 2020*

Citation:
Chaiyarit S and Thongboonkerd V (2020) Mitochondrial Dysfunction and Kidney Stone Disease. Front. Physiol. 11:566506. doi: 10.3389/fphys.2020.566506

INTRODUCTION

Mitochondrion is a unique and dynamic intracellular organelle that varies in shape, size, and number among various cell types. It is the only subcellular organelle that has double membranes and contains its own genome. Unlike nuclear DNA, mitochondrial DNA (mtDNA) is circular double-stranded DNA that is not protected by histones (Sharma and Sampath, 2019; Yan et al., 2019). Generally, mitochondria are the foremost generator of cellular energy in human cells by converting oxygen and nutritional elements to adenosine triphosphate (ATP) *via* oxidative phosphorylation pathway. Tricarboxylic acid (TCA) cycle (Kreb's cycle or citric acid cycle) and electron transport chain (ETC) are the two major metabolic processes that play pivotal roles in this energy production mechanism (Brookes et al., 2004; Pieczenik and Neustadt, 2007). Additionally, mitochondria also involve regulation of cellular oxidative stress, calcium homeostasis, apoptosis-signaling pathway, and aging processes in all cells and tissues (Bhargava and Schnellmann, 2017; Duann and Lin, 2017). Mitochondrial dysfunction, therefore, can lead to development of various metabolic diseases and other disorders, such as diabetes (Forbes and Thorburn, 2018; Ducasa et al., 2019), obesity (Song et al., 2017; Schottl et al., 2020), aging (Wojtovich et al., 2012; Giorgi et al., 2018; Jang et al., 2018; Son and Lee, 2019), cancers (Anderson et al., 2018; Neagu et al., 2019; Missiroli et al., 2020), myopathies (Walters et al., 2012; Apostolopoulou et al., 2015), and neurodegenerative disorders (Muller et al., 2018; Rango and Bresolin, 2018; Takahashi and Takahashi, 2019). Interestingly, kidney stone disease

(nephrolithiasis or urolithiasis) has been shown to be associated, either directly or indirectly, with mitochondrial dysfunction (Cao et al., 2004; Williams et al., 2016; Patel et al., 2018; Dominguez-Gutierrez et al., 2020).

This review highlights the important roles of mitochondria in renal cell functions and emphasizes associations between mitochondrial dysfunction and the pathophysiology of kidney stone disease as well as the potential for the novel approach to prevent kidney stone formation by preservation of mitochondrial functions.

ROLES OF MITOCHONDRIA IN RENAL CELL FUNCTIONS

The kidney is the second organ after the heart that contains the largest number of mitochondria per cell (Wang et al., 2010; Forbes, 2016). This organ and its related urinary tract system require sufficient energy for body homeostasis, blood filtration, nutrient reabsorption, regulation of body fluid and electrolyte, acid-base balance, and blood pressure regulation (Bhargava and Schnellmann, 2017). The most important source of cellular energy is generated by mitochondria, which are the crucial intracellular organelles to support the renal cell functions. In particular, proximal renal tubular cells are the mitochondria-rich cells that have higher density of mitochondria than other cells lining along the nephron because they require much more energy for their functions, including but not limited to reabsorption of water, glucose, ions, and nutrients (Forbes, 2016). Also, intercalated cells of the collecting ducts are the other mitochondria-rich cells playing roles in acid-base balance and regulation of sodium, chloride, and potassium transports (Roy et al., 2015).

Energy Production

ATP molecules are generated mainly by electron transfer across the mitochondrial inner membrane (MIM) *via* ETC during oxidative phosphorylation. In addition, TCA cycle also plays pivotal roles in mitochondrial energy production (Forbes, 2016). The TCA cycle produces the following coenzymes in mitochondrial matrix, including three molecules of nicotine adenine dinucleotide (NADH) and one molecule of flavin adenine dinucleotide ($FADH_2$), from acetate in the form or acetyl coenzyme A (acetyl CoA), which is converted from pyruvate *via* pyruvate dehydrogenase after glycolysis of one molecule of glucose (Pieczenik and Neustadt, 2007; Ralto et al., 2020). In addition to glycolysis, fatty acid oxidation is another mechanism that can produce even more acetyl CoA molecules for entering into the TCA cycle. As a result, fatty acid oxidation generates more NADH and $FADH_2$ molecules compared to glycolysis (Sharpe and McKenzie, 2018). Electrons from NADH and $FADH_2$ are transferred to NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II), respectively, of ETC in the MIM. The electrons were then shuttled to ubiquinol-cytochrome c reductase (complex III) by ubiquinone (coenzyme Q10; CoQ10) and transferred to cytochrome c oxidase (COX; complex IV) by cytochrome c (Bhargava and Schnellmann, 2017). During electron transfer through the ETC in the MIM, protons are actively pumped by complexes I, III, and IV through mitochondrial intermembrane space. Finally, ATP synthase (complex V) uses excess protons in mitochondrial intermembrane space to phosphorylate adenosine diphosphate (ADP) to ATP (Bhargava and Schnellmann, 2017). The requirement for ATP in the kidney is cell type-specific as tubular cells need high energy to mediate the active transport function inside the renal cortex. In contrast, cells in the glomerular segment require lower energy for filtration and other passive processes. Overall, a large number of mitochondria and their high activities are required by renal tubular cells to maintain several renal functions.

Regulation of Oxidative Stress

Oxidative stress is mostly induced by excessive cytoplasmic and mitochondrial reactive oxygen species (ROS) emission (Khand et al., 2002; Duann and Lin, 2017). Electron transfer at complexes I and III has been proposed as the main source of ROS overproduction (Ray et al., 2012). Mitochondria can consume oxygen for ATP production, leading to generation of ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). These ROS are important for cellular signaling pathways as well as cell cycles (Deng et al., 2003; Chang et al., 2005; An et al., 2013), apoptosis (An et al., 2013), protein kinases, and protein phosphatase (Brookes et al., 2002). Under physiologic condition, mitochondria have highly efficient antioxidant systems to keep the balance with ROS (Stowe and Camara, 2009). For example, superoxide dismutase rapidly reduces O_2^- to H_2O_2 faster than the rate of O_2^- production (Bhargava and Schnellmann, 2017). Subsequently, glutathione peroxidase completes the reduction processes by converting H_2O_2 to water (Fernandez-Checa et al., 1998; Ribas et al., 2014). During oxidative stress (pathological condition), excessive ROS emission occurs because the mitochondrial scavenging system is overwhelmed by the perpetual increase in ROS production, the so-called ROS-induced ROS release (RIRR; Zorov et al., 2014). As a result, mitochondrial functions are altered, leading to mtDNA damage and oxidative modifications of mitochondrial proteins and enzymes in TCA cycle and ETC (Khand et al., 2002; Bhargava and Schnellmann, 2017; Duann and Lin, 2017).

In oxidative stress condition, the nuclear factor erythroid 2-related factor 2 (NRF2) can trigger transcription factors of genes encoding antioxidant enzymes to cope with the ROS overproduction (Ruiz et al., 2013). This underscores the important regulatory roles of the mitochondrial antioxidant systems to maintain intracellular ATP production for all cellular events and to preserve mitochondrial functions. In particular, glutathione redox cycle is the critical antioxidant mechanism found in

Abbreviations: Acetyl CoA, acetyl coenzyme A; ADP, adenosine diphosphate; ATP, adenosine triphosphate; BNIP3, Bcl-2 interacting protein 3; CaOx, calcium oxalate; CypD, cyclophilin D; ETC, electron transport chain; $FADH_2$, flavin adenine dinucleotide; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; mGSH, mitochondrial glutathione; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; mtDNA, mitochondrial DNA; NADH, nicotine adenine dinucleotide; O_2^-, superoxide; ROS, reactive oxygen species; TCA, tricarboxylic acid.

cytoplasm and intracellular organelles, such as nucleus, endoplasmic reticulum, and mitochondria (Ribas et al., 2014; Reiter et al., 2018). Mitochondrial glutathione (mGSH) is the reduced form of glutathione found in mitochondrial matrix and can be oxidized to glutathione disulfide (GSSG) by superoxide anions (Fernandez-Checa et al., 1998; Ribas et al., 2014). GSSG can be reversed to mGSH by glutathione reductase that requires NADPH from the pentose phosphate pathway (Baudouin-Cornu et al., 2012; Lushchak, 2012; Bajic et al., 2019). These two mechanisms are the pivotal processes for preventing oxidative stress and preserving mitochondrial functions.

Regulation of Apoptosis

Mitochondria have been found to also involve program cell death or apoptosis (Scatena, 2012). The apoptotic cell death is considered when the cell morphology/biology is changed with membrane blebbing, cell shrinkage, and nuclear DNA fragments (Indran et al., 2011). Eventually, apoptotic cells are eliminated by neighboring and/or immune cells to avoid tissue inflammation and damage. Apoptotic cell death mechanism has two main pathways, intrinsic (*via* mitochondria) and extrinsic (*via* death receptors) pathways (Grancara et al., 2016; Abate et al., 2020). However, activation of caspases is the common and key final process that both pathways share together. Caspases are classified into two groups, based on their activities, as the initiator caspases (caspase-2, -8, -9, and -10) and the effector caspases (caspase-3, -6, and -7).

In the intrinsic pathway, mitochondrial cytochrome c is released to the cytoplasm and binds with apoptotic protease activating factor 1 (APAF-1) and ATP, resulting in recruitment of procaspase-9 to form an apoptosome, in which procaspase-9 is cleaved to caspase-9. The caspase-9 then converts procaspase-3 to effector caspase-3, resulting in the completion of cellular apoptosis (Pradelli et al., 2010). For the extrinsic pathway, extracellular ligands bind to the death receptors, leading to formation of death-inducing signaling complex that then cleaves procaspase-8 to caspase-8 (Franklin, 2011). The caspase-8 can directly stimulate the effector caspase-3 that subsequently degrades a variety of proteins during apoptosis. Moreover, the effector caspase-8 has been reported to induce cytochrome c release from mitochondria by increasing permeability of mitochondrial outer membrane (MOM) (Elmore, 2007; Indran et al., 2011). Using both intrinsic and extrinsic pathways, mitochondria thus serve as the important intracellular organelles for regulation of the apoptotic cell death.

PREVIOUS INVESTIGATIONS OF MITOCHONDRIA IN KIDNEY STONE DISEASE

Previous studies have shown that the pathogenic processes of kidney stone disease are associated with oxidative stress condition (Hirose et al., 2010; Niimi et al., 2012). The involvement of oxidative stress in kidney stone disease has been found in various *in vitro* studies (Chaiyarit and Thongboonkerd, 2012; Peerapen et al., 2018) and animal models (Hirose et al., 2010; Niimi et al., 2014) as well as stone formers (patients with kidney stones) (Ma et al., 2014; Ceban et al., 2016). Such oxidative induction leads to several downstream cascades, particularly inflammatory response and tissue injury (Williams et al., 2016; Yasui et al., 2017; Dominguez-Gutierrez et al., 2020). More importantly, there is increasing evidence demonstrating that the tissue injury induced by oxidative stress enhances retention of the causative crystals [especially calcium oxalate (CaOx)] inside renal tubules and/or kidney interstitium (parenchyma) that is one of the crucial steps for kidney stone formation (Cao et al., 2004; Khan, 2013, 2014). While a number of reports have shown overproduction of ROS in renal tubular epithelial cells followed by cellular injury, many antioxidant compounds have been introduced to cope with such oxidative stress, to reduce cellular/tissue injury, and to inhibit kidney stone formation (Muthukumar and Selvam, 1998; Itoh et al., 2005; Holoch and Tracy, 2011; Fishman et al., 2013; Zhai et al., 2013; Yang et al., 2016; Zeng et al., 2019). Because cellular oxidation is mostly associated with mitochondrial activities, mitochondria are thus directly involved in such cellular oxidative stress.

Calcium is the most common cation that is precipitated in the urine as the crystalline forms with other anions, especially oxalate and phosphate. Such calcium-containing crystals are commonly found in the urine of kidney stone formers (Yasui et al., 2017). Among all chemical types of kidney stones, CaOx is the most common one found worldwide (Vinaiphat et al., 2017; Vinaiphat and Thongboonkerd, 2017). After crystallization, CaOx crystals can adhere on the surface of renal tubular cells and are then internalized into the cells *via* macropinocytosis (Kanlaya et al., 2013) for subsequent elimination through degradation and/or dissolution (Chaiyarit et al., 2016). The end products of such elimination processes are free calcium and oxalate ions. When the intracellular calcium level is changed, mitochondria play role as one among other mechanisms to regulate intracellular calcium homeostasis. Normally, calcium can promote mitochondrial energy production. On the other hand, calcium overload may cause mitochondrial dysfunction and ROS overproduction (Brookes et al., 2004; Muller et al., 2018). The excess ROS can cause mtDNA damage followed by alterations in mitochondrial fission/fusion process, leading to cellular injury, apoptosis, inflammatory response, and finally the disease progression (Brookes et al., 2004; Suarez-Rivero et al., 2016; Srinivasan et al., 2017; Muller et al., 2018; Yan et al., 2019).

Mitochondrial dysfunction has been recognized as one of the important keys in the etiology of kidney stone disease nearly four decades ago (Laxmanan et al., 1986; Harrison et al., 1988). Calcium dense deposits have been demonstrated inside the enlarged mitochondria in renal tubular cells of the stone formers by electron microscopy without crystalline structure observed (Harrison et al., 1988). Several other investigations have also provided evidence showing the ability of mitochondria to bind with oxalate and CaOx crystals (Laxmanan et al., 1986; Govindaraj and Selvam, 2001, 2002; Selvam and Kalaiselvi, 2003; Hirose et al., 2012; Kohri et al., 2012; Roop-Ngam et al.,

2012). The binding mechanism between mitochondria and CaOx crystals has been suggested to be mediated through peroxidation of mitochondrial proteins and lipids, which further promote nucleation and aggregation of CaOx crystals (Govindaraj and Selvam, 2001). The involvement of mitochondria in early phase of kidney stone formation has gained a wide attention because mitochondrial proteins have been found in stone matrices (Govindaraj and Selvam, 2001, 2002) and the mitochondrial fragments have been found together with the crystals along distal renal tubular lumens, suggesting their roles in crystal nucleation (Hirose et al., 2012).

Although mitochondria have been found to directly interact with oxalate ion and CaOx crystals, previous investigations of mitochondria have focused only to their roles in regulation of oxidative stress and tissue injury that commonly occur in kidney stone disease. CaOx crystals have been found to induce oxidative stress in renal tubular cells leading to mitochondrial dysfunction and renal cell injury (Khan, 2013, 2014). Renal tubular cell injury and the defective mitochondria and other intracellular organelles are evidence of aggravated CaOx crystal retention inside the renal tissue, which is considered as one of the important steps for kidney stone development (Govindaraj and Selvam, 2002; Yasui et al., 2017). Following this line of investigations, several previous studies on kidney stone disease thus explored the effects of mitochondrial injury, ROS overproduction, loss of the mitochondrial membrane potential, and mitochondrial swelling (Jonassen et al., 2005; Mcmartin, 2009; Chaiyarit and Thongboonkerd, 2012; Niimi et al., 2012; Yasui et al., 2017; Peng et al., 2019; Wu et al., 2019). Moreover, CaOx crystals can induce production of mitochondrial O_2^- that further activates cyclophilin D (CypD), which is a component of mitochondrial permeability transition pore (mPTP) (Niimi et al., 2012). CypD plays a role in opening such pore and thus affects the permeability of mitochondria at MIM and MOM (Niimi et al., 2012). Additionally, CaOx induces cytochrome c release to cytoplasm for further activation of caspases and related signaling pathways, resulting in apoptosis of renal tubular cells (Jonassen et al., 2003; Niimi et al., 2012). On the other hand, mGSH maintains the mitochondrial integrity and functions, and can also reduce oxalate deposition in hyperoxaluria condition (Muthukumar and Selvam, 1998). *Vice versa*, mGSH reduction can induce mitochondrial dysfunction and may contribute to the development of CaOx kidney stones (Muthukumar and Selvam, 1998).

Our previous mitochondrial proteome study has highlighted the response of renal tubular epithelial cells of the distal nephron segment to CaOx crystals (Chaiyarit and Thongboonkerd, 2012). Using two-dimensional gel electrophoresis followed by quadrupole time-of-flight tandem mass spectrometry (Q-TOF MS/MS), a total of 15 mitochondrial proteins were identified to have differential expression levels after the distal renal tubular cells were exposed to CaOx crystals. Among these, proteins that played roles in maintaining mitochondrial functions (i.e., pyruvate dehydrogenase, ATP synthase, and NADH dehydrogenase) and cell death (ezrin) were increased (Chaiyarit and Thongboonkerd, 2012). In combination with comprehensive bioinformatics analysis of other large proteome datasets together with functional validation, the additional results indicate the association between mitochondrial dysfunction and oxidative stress-induced renal tubular cell injury (Peerapen et al., 2018).

Mitochondria have also been investigated in Randall's plaque model of kidney stone pathogenesis. Mitochondrial dysfunction has been found to be related not only to renal tubular injury but also to the impaired immune response and inflammation by decreasing monocytes' mitochondrial functions in the CaOx stone formers, leading to the decline of crystal elimination that further enhances tissue inflammation (Williams et al., 2016; Patel et al., 2018; Dominguez-Gutierrez et al., 2020). Progressive tissue inflammation together with supersaturation of calcium phosphate then induces Randall's plaque formation and finally kidney stone development (Williams et al., 2016; Patel et al., 2018; Dominguez-Gutierrez et al., 2020). To cope with the impaired immune response and tissue inflammation as well as the interstitial plaque development, various antioxidants or free radical scavengers (Muthukumar and Selvam, 1998; Kohri et al., 2012; Zhai et al., 2013; Aggarwal et al., 2016; Chhiber et al., 2016) and polysaccharide compounds (Veena et al., 2007; Sun et al., 2016; Guo et al., 2018; Sun et al., 2019) have been shown to serve as the therapeutic/preventive strategies to rescue/prevent kidney stone formation.

MECHANISMS FOR THE INVOLVEMENT OF MITOCHONDRIAL DYSFUNCTION IN PATHOPHYSIOLOGY OF KIDNEY STONE DISEASE

Oxalate and calcium can alter mitochondrial activities, leading to changes in metabolic status that may induce loss or alterations of mitochondrial functions on energy production, ROS regulation, and intracellular calcium homeostasis, all of which affect mitochondrial biogenesis (Veena et al., 2008; Hirose et al., 2010; Niimi et al., 2012; Sun et al., 2017). The dynamic processes between mitochondrial fusion and fission generate various by-products, most of which are mitochondrial fragments and ROS (Aparicio-Trejo et al., 2018; Janikiewicz et al., 2018; Geto et al., 2020). As aforementioned, several lines of evidence have suggested the involvement of mitochondrial dysfunction in the initial phase of kidney stone disease. There are three main mechanisms proposed for the involvement of mitochondrial dysfunction in the pathophysiology of kidney stone disease.

Mechanism I

Mitochondrial dysfunction can increase retention of CaOx crystals inside renal tubular lumens of the distal nephron segment. These stagnant crystals subsequently form the stone nidus, which is the central part of the stone generated from crystal aggregates that finally become the macroscopic stone. The loss or defect of mitochondrial energy production can enhance metabolic process in TCA cycle and ETC, resulting in the increase of ROS production and reduction of antioxidant enzymes (Brookes et al., 2004; Chaiyarit and Thongboonkerd, 2012; Peerapen et al., 2018). Consequently, the excess mitochondrial

free radicals can damage mitochondrial membranes (MIM and MOM). As a result, mitochondrial ROS, cytochrome c, calcium, and other proinflammatory factors are further released to the cytoplasm (Cao et al., 2016; Fong-Ngern et al., 2017). High level of cytoplasmic ROS can induce lipid peroxidation, which damages cell membranes and further enhances crystal deposition on apical surfaces of renal tubular cells (Cao et al., 2016; Fong-Ngern et al., 2017). Additionally, apoptotic signaling cascades are activated by cytochrome c that also upsurges renal tubular cell injury, leading to crystal adhesion and intrarenal crystal retention (Cao et al., 2016; Fong-Ngern et al., 2017). The accumulated crystals can be further enlarged and self-aggregated, leading to the stone formation (**Figure 1**).

Mechanism II

Mitochondrial dysfunction causes the release of cytochrome c and induces apoptotic cell death, associated with cell shrinkage, blebbing of plasma membranes, organelle condensation, and fragmentation. Thereafter, apoptotic bodies, cell debris, and fragmented subcellular organelles are released into tubular lumen and then bind with oxalate and CaOx crystals as shown by several studies (Govindaraj and Selvam, 2001, 2002; Selvam and Kalaiselvi, 2003; Hirose et al., 2012; Kohri et al., 2012). Also, fragmented mitochondria and mitochondrial proteins (i.e., 48-kDa protein) have been found in the stone core matrices and thus may get involved in the stone nidus formation (Govindaraj and Selvam, 2001, 2002). These mitochondrial components, membrane fragments, and other cellular debris can directly serve as the raw materials for the stone nidus formation in the distal nephron segment and further promote crystal nucleation, growth, and aggregation. The large aggregates may obstruct tubular lumen and/or migrate (by renal tubular fluid flow) to the calyx and pelvis to form the stone (Evan, 2010; Khan et al., 2016) (**Figure 2**).

Mechanism III

Mitochondrial dysfunction can initiate interstitial stone formation (Randall's plaque model). In oxidative stress condition, mitochondrial damage can lead to disruption of mitochondrial membrane integrity followed by the release of ROS, mtDNA, ATP, and fragmented mitochondria to the cytoplasm. These components can then induce secretion of the proinflammatory cytokines and trigger the inflammatory cascades (West, 2017). The proinflammatory cytokines that are secreted in response to mitochondrial damage also recruit various immune cells, including tissue macrophages, to this interstitial locale. Migration of these inflammatory cells can cause tissue inflammation (Singhto et al., 2013, 2018). Moreover, the renal interstitium is commonly supersaturated with calcium phosphate, which is another common crystalline compound found in kidney stones. Together with tissue inflammation, Randall's plaque rich with calcium phosphate starts to form (Khan, 2013, 2014). Some of these plaques can erode into the urinary space or renal pelvis, where supersaturation of CaOx is very common in the stone formers. At this locale (mostly lateral to the renal papilla), Randall's plaque can serve as the nidus or stem for further development or growth of CaOx stone (Khan et al., 2016; Bird and Khan, 2017; Wiener et al., 2018) (**Figure 3**).

CONCLUSIONS AND PERSPECTIVES

In summary, mitochondria may be considered as the central intracellular organelles that play pivotal roles in kidney stone pathophysiology. Alterations in their main functions, including energy production and regulation of oxidative stress and intracellular calcium homeostasis, are associated with kidney tissue injury and inflammatory response, leading to CaOx crystal nucleation, growth, aggregation, and deposition that are the key processes for kidney stone formation. Furthermore, mitochondrial

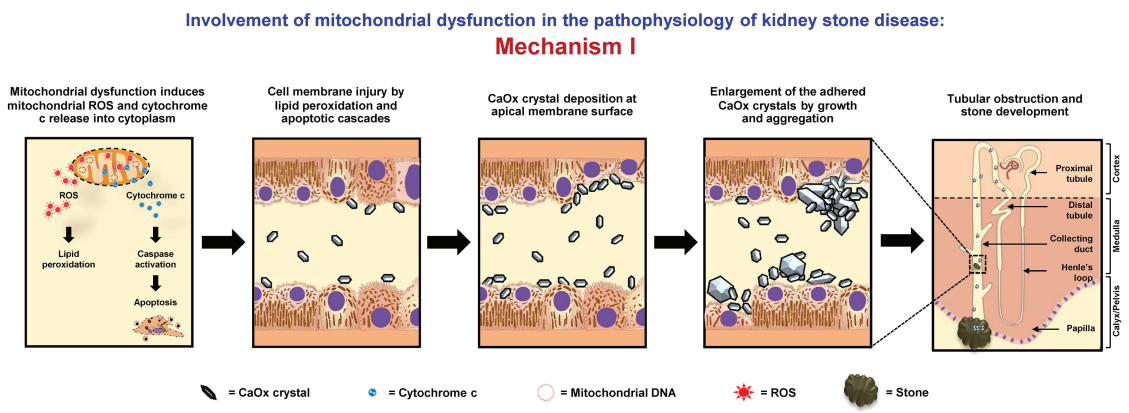


FIGURE 1 | Involvement of mitochondrial dysfunction in the pathophysiology of kidney stone disease: *Mechanism I* – Mitochondrial dysfunction induces the release of mitochondrial reactive oxygen species (ROS) and cytochrome c to cytoplasm. The lipid peroxidation and apoptotic cascades can then cause cell membrane injury that enhances calcium oxalate (CaOx) crystal deposition on apical surfaces of renal tubular cells of the distal nephron segment. The accumulated crystals can be further enlarged and self-aggregated, leading to the stone formation. The right panel shows the macroscopic diagram to demonstrate the locales (related to the nephron segments) where the proposed mechanism occurs.

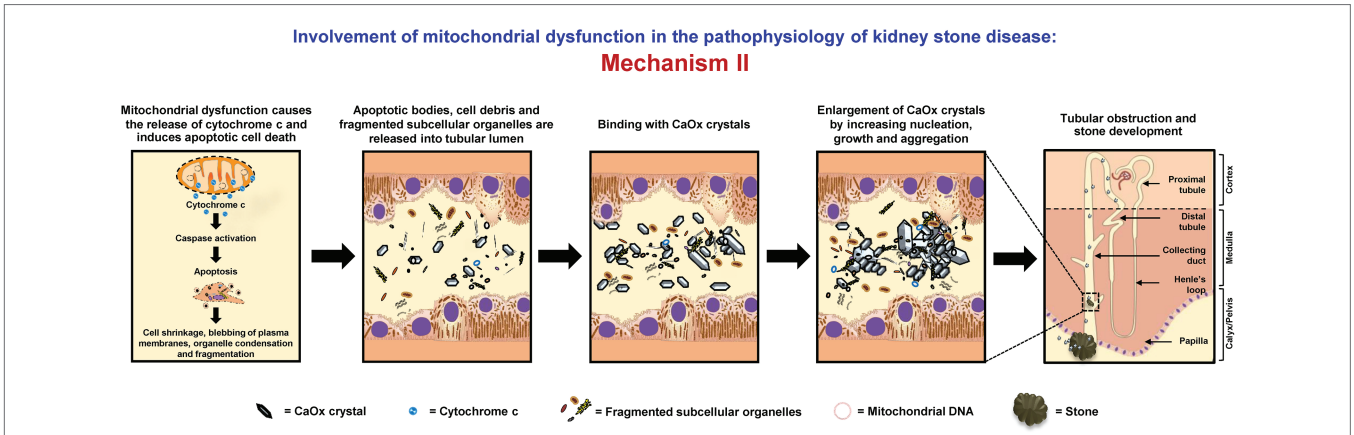


FIGURE 2 | Involvement of mitochondrial dysfunction in the pathophysiology of kidney stone disease: *Mechanism II* – Mitochondrial dysfunction causes the release of cytochrome c and induces apoptotic cell death associated with cell shrinkage, blebbing of plasma membranes, organelle condensation, and fragmentation. The resulting apoptotic bodies, cell debris, and fragmented subcellular organelles are released into tubular lumens and can then bind with CaOx crystals. Moreover, fragmented mitochondria and mitochondrial proteins together with the membrane fragments and other cellular debris can serve as the raw materials for the stone nidus formation in the distal nephron and further promote crystal nucleation, growth, and aggregation. The large aggregates may obstruct tubular lumen and/or migrate (by renal tubular fluid flow) to the calyx and pelvis to form the stone. The right panel shows the macroscopic diagram to demonstrate the locales (related to the nephron segments) where the proposed mechanism occurs.

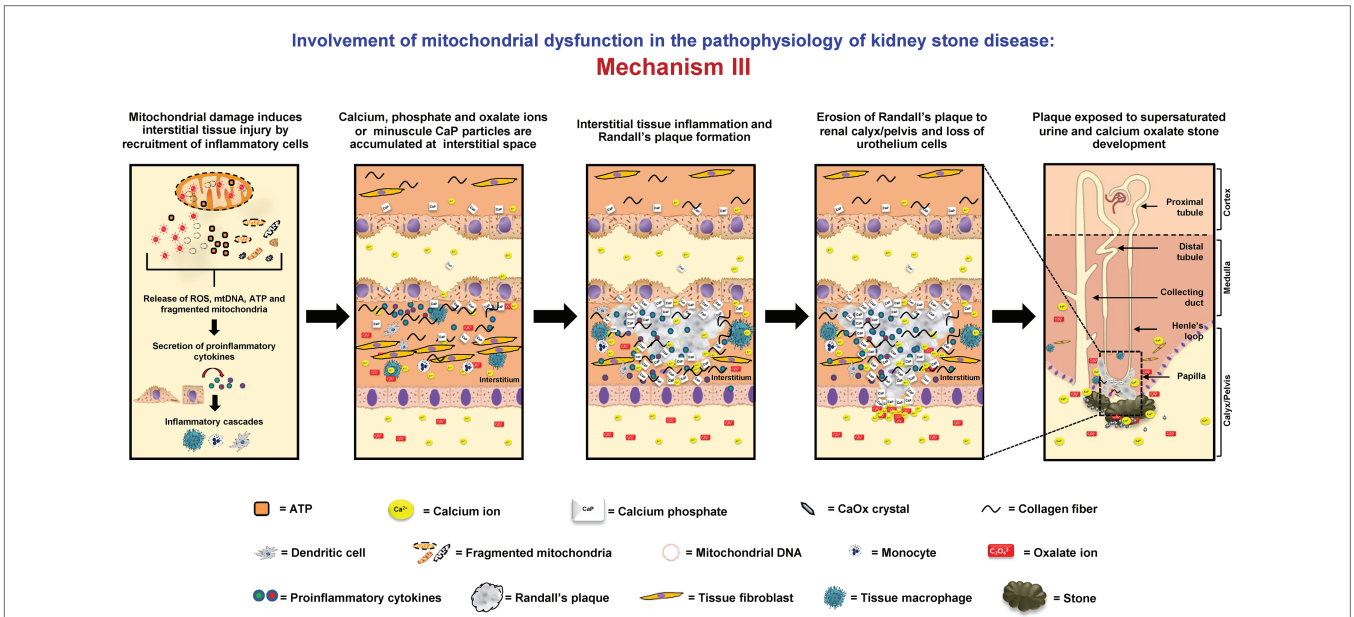


FIGURE 3 | Involvement of mitochondrial dysfunction in the pathophysiology of kidney stone disease: *Mechanism III* – Mitochondrial damage induces the disruption of mitochondrial membrane integrity followed by the release of ROS, mitochondrial DNA (mtDNA), adenosine triphosphate (ATP), and fragmented mitochondria to the cytoplasm. These components can then induce secretion of the proinflammatory cytokines, trigger the inflammatory cascades, and further enhance interstitial tissue inflammation. Together with supersaturation of calcium phosphate, which is common in the renal interstitium, Randall's plaque containing mainly calcium phosphate (hydroxyapatite) starts to form. Some of these plaques can erode into the urinary space or renal pelvis, where supersaturation of CaOx is very common in the stone formers. At this locale (mostly lateral to the renal papilla), the Randall's plaque can serve as the nidus or stem for further development or growth of CaOx stone. The right panel shows the macroscopic diagram to demonstrate the locales (related to the nephron segments) where the proposed mechanism occurs.

fragmented products and proteins can bind directly with CaOx crystals and thus play roles in the stone nidus formation. In addition to the intratubular crystal deposition and nidus formation, mitochondrial dysfunction is also associated with Randall's plaque formation by enhancing tissue inflammation and interstitial deposition of calcium phosphate (hydroxyapatite).

It should be noted that mitochondrial dysfunction alone is not sufficient to induce kidney stone formation, of which

mechanisms are multifactorial. For example, mtDNA mutations or defective nuclear-encoded mitochondrial proteins have been extensively studied in several mitochondrial disorders, e.g., Alzheimer's disease, epilepsy, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS syndrome), etc. (El-Hattab et al., 2015; Annesley and Fisher, 2019). Nevertheless, there is no evidence that link between these mitochondrial disorders and kidney stone disease. Although, MELAS syndrome also affects acid-base balanced regulated by kidney cells, the main target of this mitochondrial disorder is the neurological system, whereas kidney stone formation requires the intrarenal microenvironment involving several factors, e.g., supersaturation of CaOx, calcium phosphate, and other causative crystalline compounds, low urinary flow, imbalance of stone inhibitors and promoters, tubular cells injury, oxidative stress, inflammatory cascades, etc. (Bird and Khan, 2017).

Based on the aforementioned pathogenic mechanisms, recovering the mitochondrial functions by antioxidants may be the effective approach for prevention of new or recurrent kidney stone formation. Although antioxidant agents have been recommended to preserve mitochondrial activities and to antagonize ROS overproduction in various diseases, their efficacy and adverse events are still ambiguous and need to be further elucidated. Additionally, previous evidence has demonstrated that CypD (Duann and Lin, 2017) and Bcl-2 interacting protein 3 (BNIP3) (Chaanine et al., 2016; Peng et al., 2019) are related to mitochondrial dysfunction *via* alterations of mitochondrial membrane activities and death signaling release, respectively. To recover the mitochondrial functions and to reduce oxidative stress, inhibition of CypD activation using cyclosporin A (Duann and Lin, 2017) and N-methyl-4-isoleucine cyclosporine (Niimi et al., 2014) has been reported. In addition, expression and translocation of BNIP3 to mitochondria have been reported as the cell death regulatory factors for mitochondrial dysfunction (Zhang and Ney, 2009). This protein also involves mitochondrial membrane potential, mitochondrial transition pore forming, oxidative stress, calcium homeostasis, and inflammation in various cell types (Gao et al., 2020). Therefore, therapeutic application should be considered to combine antioxidants with other promising compounds (i.e., to inhibit activation of CypD, BNIP3 and other related molecules) for prevention of new and/or recurrence stone formation in the future. More importantly, their efficacies and adverse events must be evaluated in large cohorts.

Although the roles for mitochondrial dysfunction related to oxidative stress and CaOx crystal deposition have been well documented, the roles for excessive calcium that is also common in the stone formers (Canales et al., 2010; Chutipongtanate et al., 2012) may be overlooked. Recently, calcium is recognized as a mitochondrial regulator involving several steps of energy production (Gincel et al., 2001; Calderon-Cortes et al., 2008; Pivovarova and Andrews, 2010; Kaufman and Malhotra, 2014; Ummarino, 2017; Ham et al., 2019; Lambert et al., 2020). On the other hand, mitochondria also play roles in regulation of calcium homeostasis (Gincel et al., 2001; Calderon-Cortes et al., 2008; Pivovarova and Andrews, 2010; Kaufman and Malhotra, 2014; Ummarino, 2017; Ham et al., 2019; Lambert et al., 2020). A previous study on ethylene glycol induced kidney stone disease in rats has shown that only CaOx crystals, but not oxalate ion alone, could weaken mitochondrial functions (Mcmartin and Wallace, 2005). Nevertheless, the association among mitochondrial dysfunction, intracellular or mitochondrial calcium concentration, and the stone pathogenesis remains unclear and should be further elucidated. Having done so, the findings to be obtained may lead to the new strategy to cope with mitochondrial dysfunction during the stone development and ultimately to efficient prevention of kidney stone formation.

AUTHOR CONTRIBUTIONS

SC and VT drafted the manuscript, read and approved the final manuscript, and are responsible for all aspects of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Mahidol University research grant and the Thailand Research Fund (IRN60W0004).

REFERENCES

Abate, M., Festa, A., Falco, M., Lombardi, A., Luce, A., Grimaldi, A., et al. (2020). Mitochondria as playmakers of apoptosis, autophagy and senescence. *Semin. Cell Dev. Biol.* 98, 139–153. doi: 10.1016/j.semcdb.2019.05.022

Aggarwal, D., Gautam, D., Sharma, M., and Singla, S. K. (2016). Bergenin attenuates renal injury by reversing mitochondrial dysfunction in ethylene glycol induced hyperoxaluric rat model. *Eur. J. Pharmacol.* 791, 611–621. doi: 10.1016/j.ejphar.2016.10.002

An, J. J., Shi, K. J., Wei, W., Hua, F. Y., Ci, Y. L., Jiang, Q., et al. (2013). The ROS/JNK/ATF2 pathway mediates selenite-induced leukemia NB4 cell cycle arrest and apoptosis in vitro and in vivo. *Cell Death Dis.* 4:e973. doi: 10.1038/cddis.2013.475

Anderson, R. G., Ghiraldeli, L. P., and Pardee, T. S. (2018). Mitochondria in cancer metabolism, an organelle whose time has come? *Biochim. Biophys. Acta Rev. Cancer* 1870, 96–102. doi: 10.1016/j.bbcan.2018.05.005

Annesley, S. J., and Fisher, P. R. (2019). Mitochondria in health and disease. *Cells* 8:680. doi: 10.3390/cells8070680

Aparicio-Trejo, O. E., Tapia, E., Sanchez-Lozada, L. G., and Pedraza-Chaverri, J. (2018). Mitochondrial bioenergetics, redox state, dynamics and turnover alterations in renal mass reduction models of chronic kidney diseases and their possible implications in the progression of this illness. *Pharmacol. Res.* 135, 1–11. doi: 10.1016/j.phrs.2018.07.015

Apostolopoulou, M., Corsini, A., and Roden, M. (2015). The role of mitochondria in statin-induced myopathy. *Eur. J. Clin. Investig.* 45, 745–754. doi: 10.1111/eci.12461

Bajic, V. P., Van Neste, C., Obradovic, M., Zafirovic, S., Radak, D., Bajic, V. B., et al. (2019). Glutathione "Redox Homeostasis" and its relation to cardiovascular disease. *Oxidative Med. Cell. Longev.* 2019, 1–14. doi: 10.1155/2019/5028181

Baudouin-Cornu, P., Lagniel, G., Kumar, C., Huang, M. E., and Labarre, J. (2012). Glutathione degradation is a key determinant of glutathione homeostasis. *J. Biol. Chem.* 287, 4552–4561. doi: 10.1074/jbc.M111.315705

Bhargava, P., and Schnellmann, R. G. (2017). Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.* 13, 629–646. doi: 10.1038/nrneph.2017.107

Bird, V. Y., and Khan, S. R. (2017). How do stones form? Is unification of theories on stone formation possible? *Arch. Esp. Urol.* 70, 12–27.

Brookes, P. S., Levonen, A. L., Shiva, S., Sarti, P., and Darley-Usmar, V. M. (2002). Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic. Biol. Med.* 33, 755–764. doi: 10.1016/s0891-5849(02)00901-2

Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., and Sheu, S. S. (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.* 287, C817–C833. doi: 10.1152/ajpcell.00139.2004

Calderon-Cortes, E., Cortes-Rojo, C., Clemente-Guerrero, M., Manzo-Avalos, S., Villalobos-Molina, R., Boldogh, I., et al. (2008). Changes in mitochondrial functionality and calcium uptake in hypertensive rats as a function of age. *Mitochondrion* 8, 262–272. doi: 10.1016/j.mito.2008.04.005

Canales, B. K., Anderson, L., Higgins, L., Ensrud-Bowlin, K., Roberts, K. P., Wu, B., et al. (2010). Proteome of human calcium kidney stones. *Urology* 76, 1017.e1013–1017.e1020. doi: 10.1016/j.urology.2010.05.005

Cao, L. C., Honeyman, T. W., Cooney, R., Kennington, L., Scheid, C. R., and Jonassen, J. A. (2004). Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. *Kidney Int.* 66, 1890–1900. doi: 10.1111/j.1523-1755.2004.00963.x

Cao, Y., Liu, W., Hui, L., Zhao, J., Yang, X., Wang, Y., et al. (2016). Renal tubular injury induced by ischemia promotes the formation of calcium oxalate crystals in rats with hyperoxaluria. *Urolithiasis* 44, 389–397. doi: 10.1007/s00240-016-0876-7

Ceban, E., Banov, P., Galescu, A., and Botnari, V. (2016). Oxidative stress and antioxidant status in patients with complicated urolithiasis. *J. Med. Life* 9, 259–262.

Chaanine, A. H., Kohlbrenner, E., Gamb, S. I., Guenzel, A. J., Klaus, K., Fayyaz, A. U., et al. (2016). FOXO3a regulates BNIP3 and modulates mitochondrial calcium, dynamics, and function in cardiac stress. *Am. J. Physiol. Heart Circ. Physiol.* 311, H1540–H1559. doi: 10.1152/ajpheart.00549.2016

Chaiyarit, S., Singhto, N., and Thongboonkerd, V. (2016). Calcium oxalate monohydrate crystals internalized into renal tubular cells are degraded and dissolved by endolysosomes. *Chem. Biol. Interact.* 246, 30–35. doi: 10.1016/j.cbi.2015.12.018

Chaiyarit, S., and Thongboonkerd, V. (2012). Changes in mitochondrial proteome of renal tubular cells induced by calcium oxalate monohydrate crystal adhesion and internalization are related to mitochondrial dysfunction. *J. Proteome Res.* 11, 3269–3280. doi: 10.1021/pr300018c

Chang, H. H., Guo, M. K., Kasten, F. H., Chang, M. C., Huang, G. F., Wang, Y. L., et al. (2005). Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. *Biomaterials* 26, 745–753. doi: 10.1016/j.biomaterials.2004.03.021

Chhiber, N., Kaur, T., and Singla, S. (2016). Rottlerin, a polyphenolic compound from the fruits of Mallotus phillipensis (Lam.) Mull.Arg., impedes oxalate/calcium oxalate induced pathways of oxidative stress in male wistar rats. *Phytomedicine* 23, 989–997. doi: 10.1016/j.phymed.2016.06.005

Chutipongtanate, S., Fong-Ngern, K., Peerapen, P., and Thongboonkerd, V. (2012). High calcium enhances calcium oxalate crystal binding capacity of renal tubular cells via increased surface annexin A1 but impairs their proliferation and healing. *J. Proteome Res.* 11, 3650–3663. doi: 10.1021/pr3000738

Deng, X., Gao, F., and May, W. S. Jr. (2003). Bcl2 retards G1/S cell cycle transition by regulating intracellular ROS. *Blood* 102, 3179–3185. doi: 10.1182/blood-2003-04-1027

Dominguez-Gutierrez, P. R., Kwenda, E. P., Khan, S. R., and Canales, B. K. (2020). Immunotherapy for stone disease. *Curr. Opin. Urol.* 30, 183–189. doi: 10.1097/MOU.0000000000000729

Duann, P., and Lin, P. H. (2017). Mitochondria damage and kidney disease. *Adv. Exp. Med. Biol.* 982, 529–551. doi: 10.1007/978-3-319-55330-6_27

Ducasa, G. M., Mitrofanova, A., and Fornoni, A. (2019). Crosstalk between lipids and mitochondria in diabetic kidney disease. *Curr. Diab. Rep.* 19:144. doi: 10.1007/s11892-019-1263-x

El-Hattab, A. W., Adesina, A. M., Jones, J., and Scaglia, F. (2015). MELAS syndrome: clinical manifestations, pathogenesis, and treatment options. *Mol. Genet. Metab.* 116, 4–12. doi: 10.1016/j.ymgme.2015.06.004

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516. doi: 10.1080/01926230701320337

Evan, A. P. (2010). Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatr. Nephrol.* 25, 831–841. doi: 10.1007/s00467-009-1116-y

Fernandez-Checa, J. C., Garcia-Ruiz, C., Colell, A., Morales, A., Mari, M., Miranda, M., et al. (1998). Oxidative stress: role of mitochondria and protection by glutathione. *Biofactors* 8, 7–11. doi: 10.1002/biof.5520080102

Fishman, A. I., Green, D., Lynch, A., Choudhury, M., Eshghi, M., and Konno, S. (2013). Preventive effect of specific antioxidant on oxidative renal cell injury associated with renal crystal formation. *Urology* 82, 489.e481–489.e487. doi: 10.1016/j.urology.2013.03.065

Fong-Ngern, K., Vinaiphat, A., and Thongboonkerd, V. (2017). Microvillar injury in renal tubular epithelial cells induced by calcium oxalate crystal and the protective role of epigallocatechin-3-gallate. *FASEB J.* 31, 120–131. doi: 10.1096/fj.201600543R

Forbes, J. M. (2016). Mitochondria-power players in kidney function? *Trends Endocrinol. Metab.* 27, 441–442. doi: 10.1016/j.tem.2016.05.002

Forbes, J. M., and Thorburn, D. R. (2018). Mitochondrial dysfunction in diabetic kidney disease. *Nat. Rev. Nephrol.* 14, 291–312. doi: 10.1016/j.cca.2019.07.005

Franklin, J. L. (2011). Redox regulation of the intrinsic pathway in neuronal apoptosis. *Antioxid. Redox Signal.* 14, 1437–1448. doi: 10.1089/ars.2010.3596

Gao, A., Jiang, J., Xie, F., and Chen, L. (2020). Bnip3 in mitophagy: novel insights and potential therapeutic target for diseases of secondary mitochondrial dysfunction. *Clin. Chim. Acta* 506, 72–83. doi: 10.1016/j.cca.2020.02.024

Geto, Z., Molla, M. D., Challa, F., Belay, Y., and Getahun, T. (2020). Mitochondrial dynamic dysfunction as a main triggering factor for inflammation associated chronic non-communicable diseases. *J. Inflamm. Res.* 13, 97–107. doi: 10.2147/JIR.S232009

Gincel, D., Zaid, H., and Shoshan-Barmatz, V. (2001). Calcium binding and translocation by the voltage-dependent anion channel: a possible regulatory mechanism in mitochondrial function. *Biochem. J.* 358, 147–155. doi: 10.1042/0264-6021:3580147

Giorgi, C., Marchi, S., Simoes, I. C. M., Ren, Z., Morciano, G., Perrone, M., et al. (2018). Mitochondria and reactive oxygen species in aging and age-related diseases. *Int. Rev. Cell Mol. Biol.* 340, 209–344. doi: 10.1016/bs.ircmb.2018.05.006

Govindaraj, A., and Selvam, R. (2001). Increased calcium oxalate crystal nucleation and aggregation by peroxidized protein of human kidney stone matrix and renal cells. *Urol. Res.* 29, 194–198. doi: 10.1007/s002400100177

Govindaraj, A., and Selvam, R. (2002). An oxalate-binding protein with crystal growth promoter activity from human kidney stone matrix. *BJU Int.* 90, 336–344. doi: 10.1046/j.1464-410x.2002.02849.x

Grancara, S., Ohkubo, S., Artico, M., Ciccariello, M., Manente, S., Bragadin, M., et al. (2016). Milestones and recent discoveries on cell death mediated by mitochondria and their interactions with biologically active amines. *Amino Acids* 48, 2313–2326. doi: 10.1007/s00726-016-2323-z

Guo, D., Yu, K., Sun, X. Y., and Ouyang, J. M. (2018). Structural characterization and repair mechanism of *Gracilaria lemaneiformis* sulfated polysaccharides of different molecular weights on damaged renal epithelial cells. *Oxidative Med. Cell. Longev.* 2018, 1–15. doi: 10.1155/2018/7410389

Ham, J., Lim, W., Park, S., Bae, H., You, S., and Song, G. (2019). Synthetic phenolic antioxidant propyl gallate induces male infertility through disruption of calcium homeostasis and mitochondrial function. *Environ. Pollut.* 248, 845–856. doi: 10.1016/j.envpol.2019.02.087

Harrison, D. J., Inglis, J. A., and Tolley, D. A. (1988). Percutaneous renal biopsy specimens in stone formers. *J. Clin. Pathol.* 41, 971–974.

Hirose, M., Tozawa, K., Okada, A., Hamamoto, S., Higashibata, Y., Gao, B., et al. (2012). Role of osteopontin in early phase of renal crystal formation: immunohistochemical and microstructural comparisons with osteopontin knock-out mice. *Urol. Res.* 40, 121–129. doi: 10.1007/s00240-011-0400-z

Hirose, M., Yasui, T., Okada, A., Hamamoto, S., Shimizu, H., Itoh, Y., et al. (2010). Renal tubular epithelial cell injury and oxidative stress induce calcium oxalate crystal formation in mouse kidney. *Int. J. Urol.* 17, 83–92. doi: 10.1111/j.1442-2042.2009.02410.x

Holoch, P. A., and Tracy, C. R. (2011). Antioxidants and self-reported history of kidney stones: the National Health and Nutrition Examination Survey. *J. Endourol.* 25, 1903–1908. doi: 10.1089/end.2011.0130

Indran, I. R., Tufo, G., Pervaiz, S., and Brenner, C. (2011). Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. *Biochim. Biophys. Acta* 1807, 735–745. doi: 10.1016/j.bbabio.2011.03.010

Itoh, Y., Yasui, T., Okada, A., Tozawa, K., Hayashi, Y., and Kohri, K. (2005). Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. *J. Urol.* 173, 271–275. doi: 10.1097/01.ju.0000141311.51003.87

Jang, J. Y., Blum, A., Liu, J., and Finkel, T. (2018). The role of mitochondria in aging. *J. Clin. Invest.* 128, 3662–3670. doi: 10.1172/JCI120842

Janikiewicz, J., Szymanski, J., Malinska, D., Patalas-Krawczyk, P., Michalska, B., Duszynski, J., et al. (2018). Mitochondria-associated membranes in aging and senescence: structure, function, and dynamics. *Cell Death Dis.* 9:332. doi: 10.1038/s41419-017-0105-5

Jonassen, J. A., Cao, L. C., Honeyman, T., and Scheid, C. R. (2003). Mechanisms mediating oxalate-induced alterations in renal cell functions. *Crit. Rev. Eukaryot. Gene Expr.* 13, 55–72. doi: 10.1615/critreveukaryotgeneexpr.v13.i1.50

Jonassen, J. A., Kohjimoto, Y., Scheid, C. R., and Schmidt, M. (2005). Oxalate toxicity in renal cells. *Urol. Res.* 33, 329–339. doi: 10.1007/s00240-005-0485-3

Kanlaya, R., Sintiprungrat, K., Chaiyarit, S., and Thongboonkerd, V. (2013). Macropinocytosis is the major mechanism for endocytosis of calcium oxalate crystals into renal tubular cells. *Cell Biochem. Biophys.* 67, 1171–1179. doi: 10.1007/s12013-013-9630-8

Kaufman, R. J., and Malhotra, J. D. (2014). Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics. *Biochim. Biophys. Acta* 1843, 2233–2239. doi: 10.1016/j.bbamcr.2014.03.022

Khan, S. R. (2013). Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: evidence from clinical and experimental investigations. *J. Urol.* 189, 803–811. doi: 10.1016/j.juro.2012.05.078

Khan, S. R. (2014). Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. *Transl. Androl. Urol.* 3, 256–276. doi: 10.3978/j.issn.2223-4683.2014.06.04

Khan, S. R., Pearle, M. S., Robertson, W. G., Gambaro, G., Canales, B. K., Doizi, S., et al. (2016). Kidney stones. *Nat. Rev. Dis. Primers.* 2:16008. doi: 10.1038/nrdp.2016.8

Khand, F. D., Gordge, M. P., Robertson, W. G., Noronha-Dutra, A. A., and Hothersall, J. S. (2002). Mitochondrial superoxide production during oxalate-mediated oxidative stress in renal epithelial cells. *Free Radic. Biol. Med.* 32, 1339–1350. doi: 10.1016/s0891-5849(02)00846-8

Kohri, K., Yasui, T., Okada, A., Hirose, M., Hamamoto, S., Fujii, Y., et al. (2012). Biomolecular mechanism of urinary stone formation involving osteopontin. *Urol. Res.* 40, 623–637. doi: 10.1007/s00240-012-0514-y

Lambert, J. P., Murray, E. K., and Elrod, J. W. (2020). MCUB and mitochondrial calcium uptake - modeling, function, and therapeutic potential. *Expert Opin. Ther. Targets* 24, 163–169. doi: 10.1080/14728222.2020.1732926

Laxmanan, S., Selvam, R., Mahle, C. J., and Menon, M. (1986). Binding of oxalate to mitochondrial inner membranes of rat and human kidney. *J. Urol.* 135, 862–865.

Lushchak, V. I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *J. Amino Acids* 2012, 1–26. doi: 10.1155/2012/736837

Ma, M. C., Chen, Y. S., and Huang, H. S. (2014). Erythrocyte oxidative stress in patients with calcium oxalate stones correlates with stone size and renal tubular damage. *Urology* 83, 510.e519–510.e517. doi: 10.1016/j.urology.2013.09.050

Mcmartin, K. (2009). Are calcium oxalate crystals involved in the mechanism of acute renal failure in ethylene glycol poisoning? *Clin. Toxicol.* 47, 859–869. doi: 10.3109/15563650903344793

Mcmartin, K. E., and Wallace, K. B. (2005). Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function. *Toxicol. Sci.* 84, 195–200. doi: 10.1093/toxsci/kfi062

Missiroli, S., Genovese, I., Perrone, M., Vezzani, B., Vitto, V. A. M., and Giorgi, C. (2020). The role of mitochondria in inflammation: from cancer to neurodegenerative disorders. *J. Clin. Med.* 9:740. doi: 10.3390/jcm9030740

Muller, M., Ahumada-Castro, U., Sanhueza, M., Gonzalez-Billault, C., Court, F. A., and Cardenas, C. (2018). Mitochondria and calcium regulation as basis of neurodegeneration associated with aging. *Front. Neurosci.* 12:470. doi: 10.3389/fnins.2018.00470

Muthukumar, A., and Selvam, R. (1998). Role of glutathione on renal mitochondrial status in hyperoxaluria. *Mol. Cell. Biochem.* 185, 77–84.

Neagu, M., Constantin, C., Popescu, I. D., Zipeto, D., Tzanakakis, G., Nikitovic, D., et al. (2019). Inflammation and metabolism in cancer cell-mitochondria key player. *Front. Oncol.* 9:348. doi: 10.3389/fonc.2019.00348

Niimi, K., Yasui, T., Hirose, M., Hamamoto, S., Itoh, Y., Okada, A., et al. (2012). Mitochondrial permeability transition pore opening induces the initial process of renal calcium crystallization. *Free Radic. Biol. Med.* 52, 1207–1217. doi: 10.1016/j.freeradbiomed.2012.01.005

Niimi, K., Yasui, T., Okada, A., Hirose, Y., Kubota, Y., Umemoto, Y., et al. (2014). Novel effect of the inhibitor of mitochondrial cyclophilin D activation, N-methyl-4-isoleucine cyclosporin, on renal calcium crystallization. *Int. J. Urol.* 21, 707–713. doi: 10.1111/iju.12425

Patel, M., Yarlagadda, V., Adedoyin, O., Saini, V., Assimos, D. G., Holmes, R. P., et al. (2018). Oxalate induces mitochondrial dysfunction and disrupts redox homeostasis in a human monocyte derived cell line. *Redox Biol.* 15, 207–215. doi: 10.1016/j.redox.2017.12.003

Peerapen, P., Chaiyarit, S., and Thongboonkerd, V. (2018). Protein network analysis and functional studies of calcium oxalate crystal-induced cytotoxicity in renal tubular epithelial cells. *Proteomics* 18:e1800008. doi: 10.1002/pmic.201800008

Peng, Y., Fang, Z., Liu, M., Wang, Z., Li, L., Ming, S., et al. (2019). Testosterone induces renal tubular epithelial cell death through the HIF-1alpha/BNIP3 pathway. *J. Transl. Med.* 17:62. doi: 10.1186/s12967-019-1821-7

Pieczenik, S. R., and Neustadt, J. (2007). Mitochondrial dysfunction and molecular pathways of disease. *Exp. Mol. Pathol.* 83, 84–92. doi: 10.1016/j.yexmp.2006.09.008

Pivovarova, N. B., and Andrews, S. B. (2010). Calcium-dependent mitochondrial function and dysfunction in neurons. *FEBS J.* 277, 3622–3636. doi: 10.1111/j.1742-4658.2010.07754.x

Pradelli, L. A., Beneteau, M., and Ricci, J. E. (2010). Mitochondrial control of caspase-dependent and -independent cell death. *Cell. Mol. Life Sci.* 67, 1589–1597. doi: 10.1007/s00018-010-0285-y

Ralto, K. M., Rhee, E. P., and Parikh, S. M. (2020). $NAD^{(+)}$ homeostasis in renal health and disease. *Nat. Rev. Nephrol.* 16, 99–111. doi: 10.1038/s41581-019-0216-6

Rango, M., and Bresolin, N. (2018). Brain mitochondria, aging, and Parkinson's disease. *Genes* 9:250. doi: 10.3390/genes9050250

Ray, P. D., Huang, B. W., and Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell. Signal.* 24, 981–990. doi: 10.1016/j.cellsig.2012.01.008

Reiter, R. J., Tan, D. X., Rosales-Corral, S., Galano, A., Zhou, X. J., and Xu, B. (2018). Mitochondria: central organelles for melatonin's antioxidant and anti-aging actions. *Molecules* 23:509. doi: 10.3390/molecules23020509

Ribas, V., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2014). Glutathione and mitochondria. *Front. Pharmacol.* 5:151. doi: 10.3389/fphar.2014.00151

Roop-Ngam, P., Chaiyarit, S., Pongsakul, N., and Thongboonkerd, V. (2012). Isolation and characterizations of oxalate-binding proteins in the kidney. *Biochem. Biophys. Res. Commun.* 424, 629–634. doi: 10.1016/j.bbrc.2012.07.015

Roy, A., Al-Bataineh, M. M., and Pastor-Soler, N. M. (2015). Collecting duct intercalated cell function and regulation. *Clin. J. Am. Soc. Nephrol.* 10, 305–324. doi: 10.2215/CJN.08880914

Ruiz, S., Pergola, P. E., Zager, R. A., and Vaziri, N. D. (2013). Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int.* 83, 1029–1041. doi: 10.1038/ki.2012.439

Scatena, R. (2012). Mitochondria and cancer: a growing role in apoptosis, cancer cell metabolism and dedifferentiation. *Adv. Exp. Med. Biol.* 942, 287–308. doi: 10.1007/978-94-007-2869-1_13

Schottl, T., Pachl, F., Giesbertz, P., Daniel, H., Kuster, B., Fromme, T., et al. (2020). Proteomic and metabolite profiling reveals profound structural and metabolic reorganization of adipocyte mitochondria in obesity. *Obesity* 28, 590–600. doi: 10.1002/oby.22737

Selvam, R., and Kalaiselvi, P. (2003). Oxalate binding proteins in calcium oxalate nephrolithiasis. *Urol. Res.* 31, 242–256. doi: 10.1007/s00240-003-0316-3

Sharma, P., and Sampath, H. (2019). Mitochondrial DNA integrity: role in health and disease. *Cells* 8:100. doi: 10.3390/cells8020100

Sharpe, A. J., and Mckenzie, M. (2018). Mitochondrial fatty acid oxidation disorders associated with Short-Chain Enoyl-CoA Hydratase (ECHS1) deficiency. *Cells* 7:46. doi: 10.3390/cells7060046

Singhto, N., Kanlaya, R., Nilnumkhum, A., and Thongboonkerd, V. (2018). Roles of macrophage exosomes in immune response to calcium oxalate monohydrate crystals. *Front. Immunol.* 9:316. doi: 10.3389/fimmu.2018.00316

Singhto, N., Sintiprungrat, K., and Thongboonkerd, V. (2013). Alterations in macrophage cellular proteome induced by calcium oxalate crystals: the association of HSP90 and F-actin is important for phagosome formation. *J. Proteome Res.* 12, 3561–3572. doi: 10.1021/pr4004097

Son, J. M., and Lee, C. (2019). Mitochondria: multifaceted regulators of aging. *BMB Rep.* 52, 13–23. doi: 10.5483/BMBRep.2019.52.1.300

Song, W., Owusu-Ansah, E., Hu, Y., Cheng, D., Ni, X., Zirin, J., et al. (2017). Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-associated obesity model. *Proc. Natl. Acad. Sci. U. S. A.* 114, 8596–8601. doi: 10.1073/pnas.1708037114

Srinivasan, S., Guha, M., Kashina, A., and Avadhani, N. G. (2017). Mitochondrial dysfunction and mitochondrial dynamics-the cancer connection. *Biochim. Biophys. Acta Bioenerg.* 1858, 602–614. doi: 10.1016/j.bbabio.2017.01.004

Stowe, D. F., and Camara, A. K. (2009). Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. *Antioxid. Redox Signal.* 11, 1373–1414. doi: 10.1089/ars.2008.2331

Suarez-Rivero, J. M., Villanueva-Paz, M., De La Cruz-Ojeda, P., De La Mata, M., Cotan, D., Oropesa-Avila, M., et al. (2016). Mitochondrial dynamics in mitochondrial diseases. *Diseases* 5:1. doi: 10.3390/diseases5010001

Sun, X. Y., Ouyang, J. M., Bhadja, P., Gui, Q., Peng, H., and Liu, J. (2016). Protective effects of degraded soybean polysaccharides on renal epithelial cells exposed to oxidative damage. *J. Agric. Food Chem.* 64, 7911–7920. doi: 10.1021/acs.jafc.6b03323

Sun, X. Y., Yu, K., and Ouyang, J. M. (2017). Time-dependent subcellular structure injuries induced by nano-/micron-sized calcium oxalate monohydrate and dihydrate crystals. *Mater. Sci. Eng. C Mater. Biol. Appl.* 79, 445–456. doi: 10.1016/j.msec.2017.05.081

Sun, X. Y., Zhang, H., Liu, J., and Ouyang, J. M. (2019). Repair activity and crystal adhesion inhibition of polysaccharides with different molecular weights from red algae *Porphyra yezoensis* against oxalate-induced oxidative damage in renal epithelial cells. *Food Funct.* 10, 3851–3867. doi: 10.1039/c8fo02556h

Takahashi, M., and Takahashi, K. (2019). Water-soluble CoQ10 as a promising anti-aging agent for neurological dysfunction in brain mitochondria. *Antioxidants* 8:61. doi: 10.3390/antiox8030061

Ummarino, D. (2017). Calcium: mitochondrial calcium efflux essential for heart function. *Nat. Rev. Cardiol.* 14:317. doi: 10.1038/nrcardio.2017.73

Veena, C. K., Josephine, A., Preetha, S. P., Rajesh, N. G., and Varalakshmi, P. (2008). Mitochondrial dysfunction in an animal model of hyperoxaluria: a prophylactic approach with fucoidan. *Eur. J. Pharmacol.* 579, 330–336. doi: 10.1016/j.ejphar.2007.09.044

Veena, C. K., Josephine, A., Preetha, S. P., and Varalakshmi, P. (2007). Effect of sulphated polysaccharides on erythrocyte changes due to oxidative and nitrosative stress in experimental hyperoxaluria. *Hum. Exp. Toxicol.* 26, 923–932. doi: 10.1177/0960327107087792

Vinaiphat, A., Aluksanasuwan, S., Manissorn, J., Sutthimethakorn, S., and Thongboonkerd, V. (2017). Response of renal tubular cells to differential types and doses of calcium oxalate crystals: integrative proteome network analysis and functional investigations. *Proteomics* 17:1700192. doi: 10.1002/pmic.201700192

Vinaiphat, A., and Thongboonkerd, V. (2017). Prospects for proteomics in kidney stone disease. *Expert Rev. Proteomics* 14, 185–187. doi: 10.1080/14789450.2017.1283222

Walters, A. M., Porter, G. A. Jr., and Brookes, P. S. (2012). Mitochondria as a drug target in ischemic heart disease and cardiomyopathy. *Circ. Res.* 111, 1222–1236. doi: 10.1161/CIRCRESAHA.112.265660

Wang, Z., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., et al. (2010). Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure. *Am. J. Clin. Nutr.* 92, 1369–1377. doi: 10.3945/ajcn.2010.29885

West, A. P. (2017). Mitochondrial dysfunction as a trigger of innate immune responses and inflammation. *Toxicology* 391, 54–63. doi: 10.1016/j.tox.2017.07.016

Wiener, S. V., Ho, S. P., and Stoller, M. L. (2018). Beginnings of nephrolithiasis: insights into the past, present and future of Randall's plaque formation research. *Curr. Opin. Nephrol. Hypertens.* 27, 236–242. doi: 10.1097/MNH.0000000000000414

Williams, J., Holmes, R. P., Assimos, D. G., and Mitchell, T. (2016). Monocyte mitochondrial function in calcium oxalate stone formers. *Urology* 93, 224–226. doi: 10.1016/j.urology.2016.03.004

Wojtovich, A. P., Nadtochiy, S. M., Brookes, P. S., and Nehrke, K. (2012). Ischemic preconditioning: the role of mitochondria and aging. *Exp. Gerontol.* 47, 1–7. doi: 10.1016/j.exger.2011.11.001

Wu, J., Tao, Z., Deng, Y., Liu, Q., Liu, Y., Guan, X., et al. (2019). Calcifying nanoparticles induce cytotoxicity mediated by ROS-JNK signaling pathways. *Urolithiasis* 47, 125–135. doi: 10.1007/s00240-018-1048-8

Yan, C., Duanmu, X., Zeng, L., Liu, B., and Song, Z. (2019). Mitochondrial DNA: distribution, mutations, and elimination. *Cells* 8:379. doi: 10.3390/cells8040379

Yang, X., Ding, H., Qin, Z., Zhang, C., Qi, S., Zhang, H., et al. (2016). Metformin prevents renal stone formation through an antioxidant mechanism in vitro and in vivo. *Oxidative Med. Cell. Longev.* 2016, 1–10. doi: 10.1155/2016/4156075

Yasui, T., Okada, A., Hamamoto, S., Ando, R., Taguchi, K., Tozawa, K., et al. (2017). Pathophysiology-based treatment of urolithiasis. *Int. J. Urol.* 24, 32–38. doi: 10.1111/iju.13187

Zeng, X., Xi, Y., and Jiang, W. (2019). Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review. *Crit. Rev. Food Sci. Nutr.* 59, 2125–2135. doi: 10.1080/10408398.2018.1439880

Zhai, W., Zheng, J., Yao, X., Peng, B., Liu, M., Huang, J., et al. (2013). Catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization in NRK-52E cells and the ethylene glycol induced renal stone formation in rat. *BMC Complement. Altern. Med.* 13:228. doi: 10.1186/1472-6882-13-228

Zhang, J., and Ney, P. A. (2009). Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ.* 16, 939–946. doi: 10.1038/cdd.2009.16

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* 94, 909–950. doi: 10.1152/physrev.00026.2013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership