Kidney physiology and susceptibility to acute kidney injury: implications for renoprotection

Holger Scholz[1], Felix J. Boivin[1,2], Kai M. Schmidt-Ott[1,2], Sebastian Bachmann[1], Kai-Uwe Eckardt[1], Ute I. Scholl[1] and Pontus B. Persson[1] ✉

Abstract | Kidney damage varies according to the primary insult. Different aetiologies of acute kidney injury (AKI), including kidney ischaemia, exposure to nephrotoxins, dehydration or sepsis, are associated with characteristic patterns of damage and changes in gene expression, which can provide insight into the mechanisms that lead to persistent structural and functional damage. Early morphological alterations are driven by a delicate balance between energy demand and oxygen supply, which varies considerably in different regions of the kidney. The functional heterogeneity of the various nephron segments is reflected in their use of different metabolic pathways. AKI is often linked to defects in kidney oxygen supply, and some nephron segments might not be able to shift to anaerobic metabolism under low oxygen conditions or might have remarkably low basal oxygen levels, which enhances their vulnerability to damage. Here, we discuss why specific kidney regions are at particular risk of injury and how this information might help to delineate novel routes for mitigating injury and avoiding permanent damage. We suggest that the physiological heterogeneity of the kidney should be taken into account when exploring novel renoprotective strategies, such as improvement of kidney tissue oxygenation, stimulation of hypoxia signalling pathways and modulation of cellular energy metabolism.

According to the Kidney Disease: Improving Global Outcomes clinical practice guidelines, acute kidney injury (AKI) involves a rapid deterioration of kidney function (usually occurring over a period of hours to days) that is defined by an increase in serum creatinine, a decline in urine excretion or both. Approximately 15% of adults and 25% of children admitted to hospital develop AKI[1,2]. The incidence of AKI is even higher in patients receiving intensive care (>57% in a large international study)[3]. Notably, multiple studies demonstrate that AKI is associated with an increased risk of developing chronic kidney disease (CKD)[2]. Post-AKI CKD is thought to result from maladaptive repair, which leads to fibrosis, vascular rarefaction, tubular loss, glomerulosclerosis and chronic inflammation following the acute phase of injury.

Despite decades of intensive research, the complex pathophysiology of AKI is still not well understood. Consequently, preventive and therapeutic approaches remain unsatisfactory. A major obstacle is that, rather than being a disease entity, AKI represents a highly heterogeneous group of conditions that share rapid loss of kidney function as an outcome. Serum creatinine level and urinary output, which are used for diagnosis, are neither sensitive nor specific for AKI[2], and changes in serum creatinine neither correlate closely with the severity of kidney damage nor indicate the cause of AKI[4,5].

Sepsis is a predominant cause of AKI in hospitalized patients, particularly in the intensive care unit. Other clinical conditions associated with AKI include major surgery, cardiorenal syndrome (that is, acute worsening of kidney function in the setting of heart failure), kidney hypoperfusion of other causes (for example, hypovolaemia, systemic vasodilation or increased central venous pressure (CVP)), systemic inflammation and inflammatory processes within the kidney (for example, due to pyelonephritis, acute interstitial nephritis or glomerulonephritis), exposure to nephrotoxins, postrenal urinary tract obstruction and intra-abdominal hypertension[2,6–8] (BOX 1). This broad clinical spectrum raises the question of whether different aetiologies of kidney injury activate distinct pathophysiological mechanisms or whether they share a common final pathway.

Several studies have identified nephron segment-specific gene expression changes in response to diverse stimuli that can lead to AKI, which suggests that substantially different molecular categories of AKI exist[9–11]. Even aetiologies that were traditionally thought to be

[1]*Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany.*

[2]*Molecular and Translational Kidney Research, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.*

✉*e-mail: pontus.persson@charite.de*

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Key points

- Rather than a disease entity, acute kidney injury (AKI) comprises a heterogeneous group of conditions that lead to a rapid decline in excretory kidney function.
- Distinct AKI aetiologies, including sepsis, major surgery, hypovolaemia and inflammatory processes within the kidney, are associated with complex pathophysiological processes that are characterized by distinct changes in gene expression patterns.
- The unique vascularization of the kidney is associated with regional heterogeneity of oxygen supply, which creates areas at high risk of hypoxia.
- Tubular segments differ in their ability to use substrates for ATP generation and in their energy demand, which creates further variability in their susceptibility to injury.
- Emerging renoprotective strategies address these unique susceptibilities and distinct aetiologies to improve oxygen supply, prevent mitochondrial dysfunction or regulate metabolic pathways and oxygen consumption.
- Over the next few years, pathophysiology-guided strategies are expected to translate into improvements in AKI clinical prevention, diagnosis and therapy.

related, such as ischaemia–reperfusion and volume depletion, activate functionally unrelated signal transduction pathways and trigger responses in different regions of the kidney, which suggests distinct rather than shared pathologies[12]. Ischaemia–reperfusion and lipopolysaccharide (LPS) treatment (used as an experimental model of sepsis) also induced the expression of different kidney injury markers[13]. The occurrence of different AKI pathomechanisms or markers is relevant with regard to the development of novel strategies for renoprotection. Moreover, a better understanding of its underlying mechanisms might help to improve the assessment of the clinical course of AKI and enable a diagnosis based on specific molecular pathways, rather than unspecific parameters such as serum creatinine and urinary output.

The anatomical and metabolic properties of different kidney regions might explain their varying sensitivities to damage in AKI. The capacity to concentrate urine through a countercurrent mechanism requires a highly complex kidney architecture. The kidneys are perfused with ~25% of cardiac output but the countercurrent arrangement of microvessels (that is, blood flows through arteries and veins in opposite directions) affects tissue oxygenation, which predisposes large parts of the kidney to oxygen shortage[14]. Another challenge lies in the vast energy demand of the kidneys, which increases linearly with tubular transport activity. More than 90% of ATP production in the kidney occurs through aerobic mechanisms; therefore, any disruption in the delicate balance between oxygen supply and demand increases the risk of AKI. Kidney oxygen deficiency might result from a critical reduction in kidney blood flow and/or altered intrarenal haemodynamics (for example, due to compression of the kidney microvessels as a result of tubular swelling[15], mitochondrial leakage[16] or inflammatory processes[17]).

In this Review, we integrate kidney physiology and AKI mechanisms to outline novel concepts for the refinement of diagnostic approaches and renoprotective strategies. We argue that physiological heterogeneity in tissue oxygenation, metabolism and cellular protection should guide the development of new protocols for the treatment of AKI.

Diffusional equilibrium
A condition of balanced oxygen distribution (that is, lack of partial pressure of oxygen (pO_2) heterogeneities) within a tissue.

Arterial–venous shunting
The diffusion of oxygen from arteries to veins in blood vessels that are arranged in a countercurrent.

Kidney physiological heterogeneity

Collaborative organization of the nephron segments enables normal kidney function. Proximal tubules reabsorb the bulk of filtered fluid and solutes, whereas the more distal nephron segments are important for the concentration of urine and the regulation of salt excretion. Notably, the various nephron segments use different metabolic pathways to meet their energy demands for tubular transport[18]. This heterogeneity in metabolic supply and demand may account, at least in part, for the variable regional susceptibility of kidney tubule cells to hypoxia. Moreover, the vulnerability of single nephron segments is also determined by their ability to activate cellular defence mechanisms and repair programmes. Thus, differences in the protective capacity and cellular plasticity of distinct nephron segments (for example, modulation of mitochondrial function[16]) might contribute to the kidney damage patterns caused by injurious stimuli.

Kidney oxygen supply

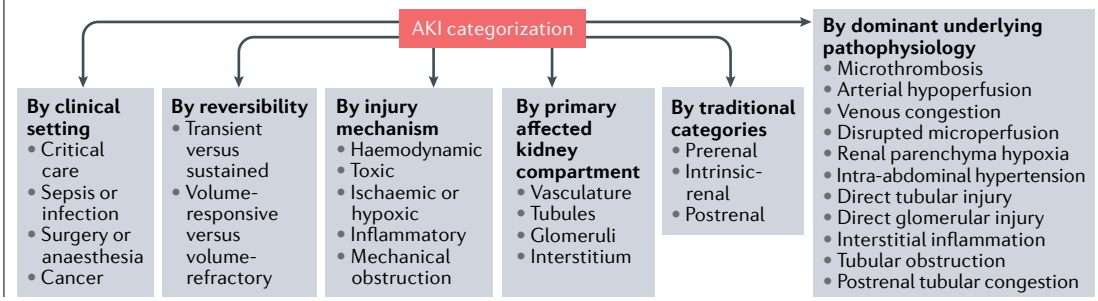
The kidney has a particularly high specific perfusion rate — kidney blood flow amounts to approximately 1.2 l/min — which is required for maintaining a high glomerular filtration rate (GFR). Consequently, despite its high metabolic rate, metabolic regulation of blood flow in the kidney is limited, which facilitates GFR maintenance but increases its predisposition to hypoxia.

Blood enters the kidney through the cortex, from where only 10% of blood vessels branch off to supply the kidney medulla, which creates a considerable gradient in the cortico-medullary partial pressure of oxygen (pO_2) (BOX 2). The very high osmotic concentration in the kidney medulla allows effective urine concentration. Low perfusion rates and the kidney vascular network arrangement enable the so-called countercurrent mechanism to prevent osmolyte loss in the medulla at the cost of low pO_2 in the deeper portions of the kidney.

Cortex. New technologies continue to advance our understanding of oxygen supply and utilization in the kidney tubule. For example, intravital confocal phosphorescence lifetime imaging microscopy in the proximal tubule of mouse kidney allows unmatched spatial resolution of pO_2 within cells[19]. This method combines oxygen quenching, which had previously been applied to the kidney[20], with high-resolution imaging, and findings from its application suggest that oxygen tension in the superficial kidney cortex is heterogeneous rather than existing at a diffusional equilibrium[21] (BOX 2). This heterogeneity in cortical pO_2 might be due to arterial–venous shunting. Cortical vessels are arranged in a countercurrent, and the larger vessels might have sufficiently small diffusion barriers to allow effective shunting of oxygen[22]. Cortical pO_2 heterogeneity might also result from oxygen consumption as the tubular fluid flows into the S_3 segment of the proximal tubule. Moreover, the high metabolic rate of the S_1 and S_2 epithelia (which is dependent on mitochondrial function)[23] requires a constant oxygen supply (BOX 2). Consequently, despite high blood flow, heterogeneity in pO_2 within the kidney cortex renders it vulnerable to hypoxia.

Box 1 | **Aetiologies of human AKI**

Acute kidney injury (AKI) is clinically defined by increases in serum creatinine or decreases in urinary output. Because AKI is a clinical syndrome rather than a distinct disease entity, the key goal of clinical evaluation of AKI is to identify and address the underlying aetiology. A multitude of approaches to categorizing AKI have been proposed, including classification by clinical setting, reversibility or response to therapy, general mechanism of injury, primary affected anatomical compartment, dominant underlying pathophysiology or traditional categories. Each of these approaches to classification has inherent problems — the categories are either too broad (for example, perioperative AKI can be caused by hypotension, hypoxia, nephrotoxic medication or sepsis), not prospective (for example, volume-responsive AKI can only be diagnosed after assessing the therapeutic response) or not mutually exclusive (for example, sustained prerenal AKI or postrenal AKI frequently cause intrinsic AKI owing to tubular injury). Given this complexity of AKI, the most appropriate approach would likely be a multidimensional classification that guides towards the most appropriate therapeutic interventions.



Outer medulla. Mitochondria in the outer medulla, which harbours the S_3 segments of the proximal tubules and the more distal portion of the medullary thick ascending limb (mTAL), are particularly efficient[24] as this region exhibits the lowest pO_2 levels in the kidney. Currently, pO_2 in the TAL segment can only be estimated through mathematical models[25]. According to such models, TALs in the outer stripe of the medulla have the lowest pO_2 in the nephron. Local pO_2 is estimated to be ~20 mmHg (BOX 2) given the high oxygen consumption of the TAL. Moreover, the vascular bed surrounding the outer medulla is unique. Vessels supplying the medulla exit the kidney cortex in bundles and are distant from the metabolically very active S_3 segments and the TAL. Thus, in the outer stripe of the medulla, the S_3 segments and the TAL rely on capillary–venous oxygen supply[26]. Furthermore, capillary density is low in the outer medulla. Consequently, diffusion distances limit the oxygen supply to the S_3 segment. Finally, arterial–venous anastomoses efficiently shunt oxygenated blood away from the medullary circulation[22].

Adding to the conundrum of limited oxygen supply to the outer medulla, this area of the kidney is prone to vascular congestion for reasons not fully understood[27]. Congestion in outer medullary vessels is a hallmark of damage in transplanted cadaveric kidneys. Of note, descending vasa recta have a rich supply of surrounding pericytes[28], which show pronounced peristaltic-like contractions to maintain flow throughout the downstream capillary network[29]. Pericytes are thought to lessen congestion by maintaining a rhythmic blood flow and, accordingly, reducing pericyte abundance experimentally enhances congestion in peritubular capillaries[29].

The high energy demands and limited oxygen supply of the TAL and S_3 proximal tubule epithelial cells therefore pose a threat to their viability. Under certain conditions, such as cardiopulmonary bypass (CPB)[30] or sepsis[31], the already low pO_2 values in portions of the outer medulla decrease to critical levels. Medullary oxygenation balance, which is a term used to describe the relationship between oxygen supply and demand, is key to understanding hypoxic damage to this region[14,32]. Damage to the S_3 nephron segment is typically observed in many forms of ischaemia (for example, in transplantation) because the S_3 segment has low anaerobic capacity (discussed in more detail later). Compared with S_3 epithelial cells, TALs have greater anaerobic ability but their transport activity is very high, which also limits cell survival in conditions of restricted oxygen supply and high transport requirements. For instance, in warm ischaemia, transport is dramatically reduced owing to energy shortage; thus, TAL cells survive better than S_3 epithelial cells. By contrast, in situations of hypoxia and high transport function (for example, seawater near-drowning), the TAL suffers greater damage than S_3 epithelia[32]. These examples highlight the importance of medullary oxygenation balance.

Inner medulla. Oxygen tension values for tubular segments other than the proximal tubule cannot be currently assessed directly. However, urinary pO_2 is commonly used to estimate the collecting duct pO_2 and serves as a measure of kidney medullary pO_2 (REFS[33,34]), given that oxygen can freely diffuse between the kidney medulla and urine. Inner medulla pO_2 is estimated at ~35 mmHg and is therefore thought to be higher than that of the outer medulla[25,35] (BOX 2). Notably, urinary pO_2 decreases within an hour in experimental sepsis, which is much earlier than changes in common kidney markers of injury, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM1; also known as HAVCR1), can be observed[33]. Thus, urinary pO_2 might have potential as an early marker in sepsis-associated AKI.

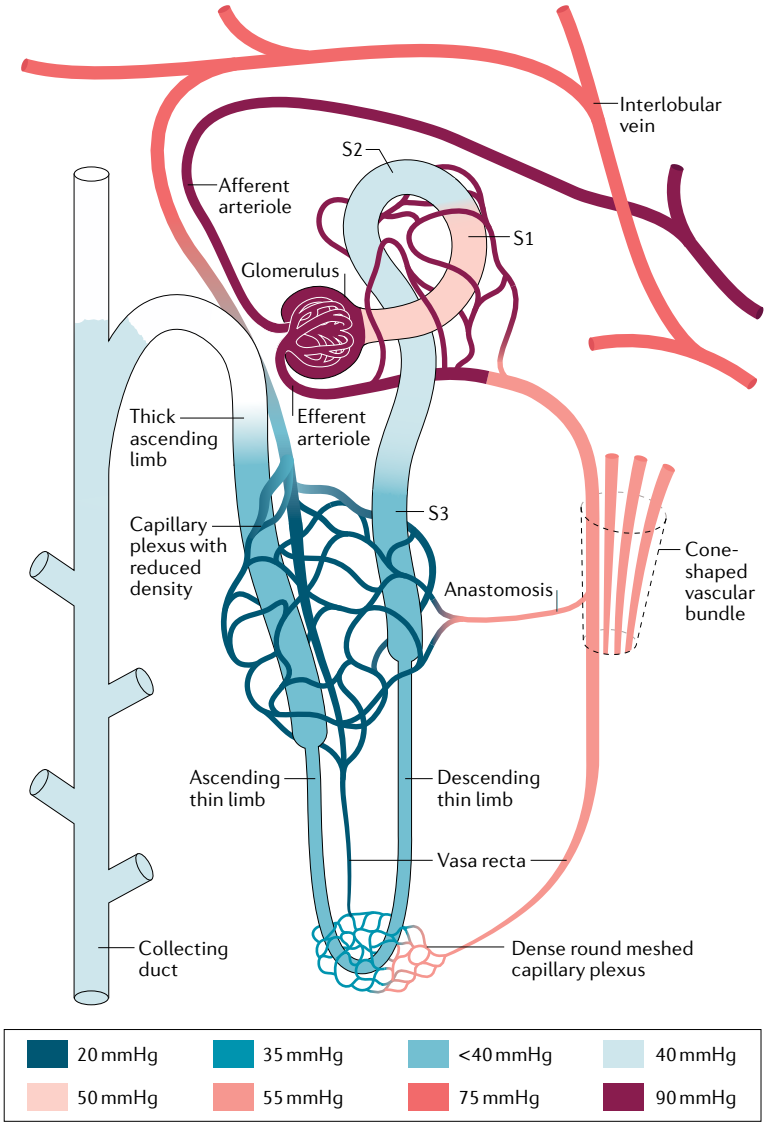
Vascular congestion
In the context of acute kidney injury, vascular congestion describes reduced kidney perfusion in critical regions due to low blood flow and erythrocyte aggregation.

Box 2 | **Regional pO_2 gradients increase the risk of hypoxic damage in the juxtamedullary nephron**

The partial pressure of oxygen (pO_2) in kidney tissue varies enormously, even within defined regions of the kidney such as the cortex. Modified blood oxygenation level-dependent (BOLD)-MRI can provide information on the heterogeneity of the oxygen supply in the kidney cortex on a large scale[164]. The regional pO_2 heterogeneity in the kidney cortex is determined by two phenomena. The tubule epithelia rely on the tubular fluid oxygen supply; therefore, oxygen consumption along the proximal tubule reduces pO_2 in downstream regions. Moreover, the diffusion distance from the supplying blood vessels to the consuming epithelium affects local pO_2.

An oxygen tension gradient occurs along the proximal tubule[19], which might account for the varying susceptibility of these cells to hypoxia. Luminal pO_2 is readily consumed by the S_1 and S_2 segments. Consequently, the downstream S_3 segment is at particular risk of hypoxic damage. The pO_2 of the S_3 segment has not yet been directly measured, but two-photon microscopy of phosphorescence lifetime might fill this gap in knowledge[21].

The broad range of pO_2 in the kidney medulla is due to the complex arrangement of tubules and vessels[35]. For this reason, estimating regional oxygen supply requires sophisticated mathematical modelling[165], which shows that the largest pO_2 gradient occurs in the outer medulla[35]. Descending vasa recta (DVR) enter the outer medulla in cone-shaped bundles, where pO_2 is very high. Much lower pO_2 values have been determined for regions distant from the DVR. Only DVR originating from the juxtamedullary glomeruli supply this region with blood and therefore only one in five DVR reach the inner medulla. To avoid severe hypoxia in the inner medulla, the DVR in the outer medulla are separate from the thick ascending limbs. These thick ascending limbs and S_3 epithelial cells are located in the outer medullary region and might consume oxygen from deeper portions of the nephron. Accordingly, the vicinity of S_3 segments has particularly low pO_2 and the main source of S_3 oxygen supply is the venous–capillary network. Given that S_3 and thick ascending limb epithelial cells have high metabolic rates, maintaining the medullary oxygenation balance can be challenging.



Vulnerability of nephron segments

The kidneys contribute to less than 1% of the total human body weight but use ~7% of the daily energy produced under resting conditions. After the heart, the kidney is the organ with the highest resting metabolic rate in healthy adults[36]. The enormous energy demand of the kidney is reflected in its mitochondrial density and oxygen consumption rate[16], which is also the second highest in the body, after the heart[37]. Most of the energy consumed by the kidney is used for reabsorbing electrolytes and nutrients from the tubular fluid into the blood and for secreting waste products from the blood into the tubular fluid. The driving force for epithelial transport is provided mainly by the activity of the Na^+–K^+–ATPase, which generates ion gradients across the cell membrane. As outlined in detail below, the intrarenal oxygenation profile, and thus the vulnerability of nephron segments to hypoxia and other damage mechanisms, depends on their epithelial transport activities. This relationship is illustrated by the results of a study performed on hospitalized patients with type 2 diabetes mellitus who were being treated with sodium–glucose cotransporter 2 (SGLT2)

inhibitors. Compared with patients who maintained kidney function, patients presenting with AKI on admission had increased serum and urine levels of the distal tubule injury marker NGAL[38]. However, KIM1, which is indicative of proximal tubule damage, was normal in patients with AKI. These results suggest that decreasing proximal tubular transport activity through SGLT2 inhibition might improve cortical oxygenation but aggravate medullary hypoxia in the setting of hypoxia[38].

Proximal tubules. Although all cells in the kidney require ATP to maintain their normal function, the biochemical pathways used for ATP synthesis vary between different cell types (FIG. 1). Approximately 70% of the glomerular filtrate and its solutes are reabsorbed in the proximal tubules, which explains the high energy demand in this nephron segment. Proximal tubules generate ATP primarily through aerobic respiration and can use a variety of substrates, including non-esterified fatty acids, glutamine, pyruvate, citrate and lactate (FIG. 1). Increased catabolism of glutamine, and consequent urinary excretion of acidic ammonium ions and uptake of bicarbonate into the blood, is an essential compensatory kidney response to metabolic acidosis[39]. In addition to the role of pyruvate as a metabolic intermediate in cellular energy homeostasis, its antioxidant and anti-inflammatory properties provide protection against AKI induced by rhabdomyolysis or sepsis in mice[40]. Several studies indicate that the kidney is unique in taking up the tricarboxylic acid (TCA) cycle intermediate citrate from the circulation, which occurs largely through reabsorption in the proximal tubules[41–43]. Citrate uptake in the kidney is substantial and contributes to ~20% of its TCA cycle activity[41,42]. The kidney also has an important role in gluconeogenesis and proximal tubule cells are the only kidney cell type that is capable of de novo synthesis of glucose, mainly by using lactate as a precursor. To avoid a futile circle of glucose synthesis and metabolism, these cells have low hexokinase activity and therefore metabolize glucose poorly. However, proximal tubule cells efficiently use β-oxidation of fatty acids as a fuel source, which yields more ATP per substrate molecule than glucose oxidation and is therefore advantageous given the high energy demand of these cells. Compared with the upstream S_1 and S_2 proximal tubule cells in the kidney cortex, the S_3 segment has slightly higher hexokinase activity and can produce ATP from glucose[44]. However, its low anaerobic glycolytic capacity, compared with more distal nephron segments, and its localization in the outer medulla with low pO_2 values, put the S_3 segment at risk of energy shortage under hypoxic conditions[44]. Overall, proximal tubules metabolize a wide range of substrates but have little capacity for glycolysis (FIG. 1).

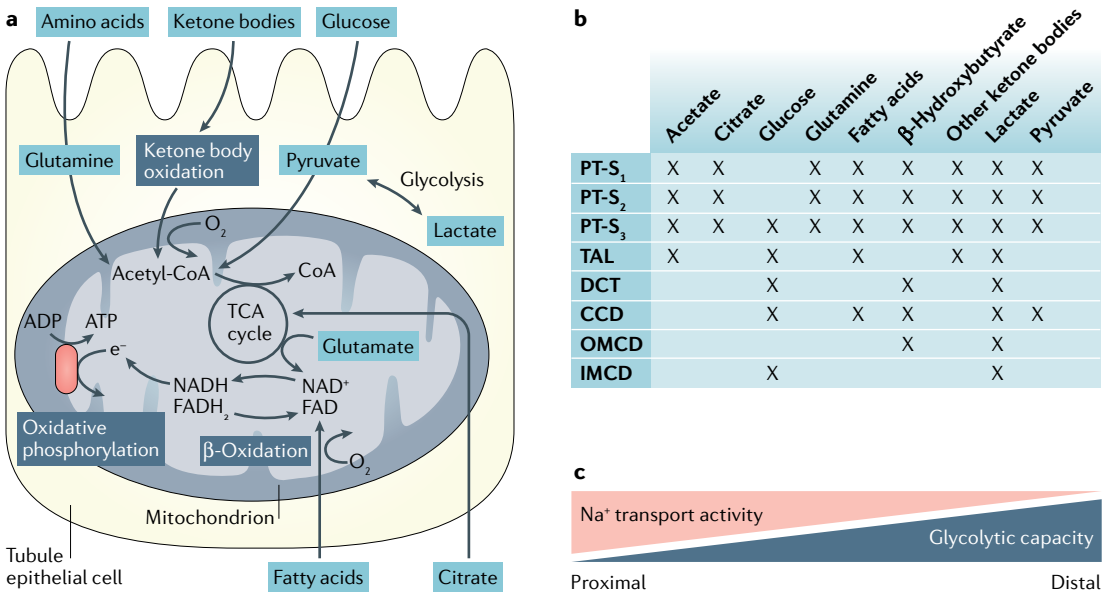


Fig. 1 | **Metabolic pathways across nephron segments. a** | This panel illustrates the metabolic pathways of kidney tubule cells. Energy sources include glucose, amino acids, fatty acids and ketone bodies, which are metabolized to acetyl-CoA before entering the tricarboxylic acid (TCA) cycle. The kidney is also able to take up the TCA intermediate citrate from the circulation[41–43], and kidney tubule cells can enzymatically convert lactate to pyruvate. **b** | Importantly, distinct nephron segments preferentially use different substrates to fulfil their energy requirements. For example, although the S_1 and S_2 segments of the proximal tubule (PT) reabsorb the bulk of glucose from the tubular fluid, they cannot use glucose as a fuel because they lack sufficient activity of hexokinase, the first enzyme in the glycolytic pathway. Furthermore, the glycolytic capacity of the S_3 segments is also low, which places this nephron segment at risk of energy shortage under hypoxic conditions. In contrast to other ketone bodies, substantial amounts of β-hydroxybutyrate are taken up by the more distal nephron segments, for which β-hydroxybutyrate serves as a preferred substrate.[44] **c** | In general, glycolytic capacity increases, whereas sodium transport activity is reduced, from the proximal to the distal parts of the nephron.CCD, cortical collecting duct; DCT, distal convoluted tubule; IMCD, inner medullary collecting duct; OMCD, outer medullary collecting duct; TAL, thick ascending limb. Panels **a** and **b** adapted with permission from REF.[162], Elsevier.

Distal nephron segments. As described above, blood supply to the medulla is scarce, which yields low pO_2 values. Therefore, glucose metabolism in the medulla relies substantially on anaerobic glycolysis. Lactate production is indicative of anaerobic glycolysis and, whereas isolated rat proximal tubules did not produce lactate under baseline conditions, considerable amounts of lactate were released in vitro from all distal nephron segments; the inner medullary collecting duct had the highest lactate production rate[45]. Moreover, pharmacological inhibition of oxidative phosphorylation with antimycin A increased lactate formation in all distal nephron segments but not in proximal tubules[45,46]. The largest increase in lactate production under anaerobic conditions occurred in the mTAL (14-fold), followed by the cortical collecting duct (eightfold) and the outer medullary collecting duct (fourfold)[45]. These findings indicate that the distal portions of the nephron can generate substantial amounts of ATP by anaerobic glycolysis, which allows them to fulfil their physiological functions under the low pO_2 conditions of the kidney medulla.

As mentioned earlier, nephron segments with high energy demand (for example, owing to extensive transport activity), critical oxygen supply, even under normal conditions, and low anaerobic reserve are particularly susceptible to kidney injury. These criteria apply primarily to the outer stripe of the outer medulla (that is, S_3 segments and mTAL). The proximal tubule relies on oxidative phosphorylation to generate appropriate amounts of ATP, whereas the glycolytic capacity of mTAL cells increases under anaerobic conditions (FIG. 1). Their higher glycolytic capacity renders mTAL cells resistant to hypoxia as long as the energy supply is sufficient for maintaining epithelial transport. In vitro studies performed on isolated perfused kidneys and in vivo animal model data indicate that tubular transport activity is the principal parameter that determines the development of mTAL injury when oxygen supply declines critically[47,48]. Thus, disturbance of the medullary oxygenation balance, which depends on kidney oxygen supply and consumption, and is therefore dependent on epithelial transport activity, has a major role in the particular vulnerability of TAL segments to hypoxic injury[32].

AKI-related metabolic changes. Enhanced glycolysis combined with mitochondrial dysfunction is a hallmark of various AKI aetiologies, including ischaemia–reperfusion injury and sepsis[40,49,50]. Under these conditions, activation of the polyol pathway generates fructose from glucose in two consecutive enzymatic reactions that involve aldose reductase and sorbitol dehydrogenase[51]. Notably, the polyol pathway is normally inactive in healthy kidneys owing to an absence of aldose reductase expression. However, aldose reductase is upregulated in response to kidney ischaemia, which increases fructose production in the kidney cortex of mice and enhances urinary fructose excretion in patients with AKI[52]. Phosphorylation of fructose by fructokinase reduces intracellular phosphate levels and enhances the activity of AMP deaminase, which in turn catalyses the conversion of AMP to inosine monophosphate (IMP), leading to the formation of uric acid and toxic advanced glycation end products. Elevated serum levels of uric acid are associated with kidney injury, likely because of tubular crystal precipitation and activation of an inflammatory response[53]. Interestingly, fructokinase-deficient mice with ischaemic AKI display lower kidney uric acid levels and milder kidney injury than wild-type mice, which indicates that this pathway has a detrimental role in this type of AKI[52,54]. The beneficial effect of fructokinase deficiency correlated with reduced kidney inflammation and oxidative stress[52].

Nutrient-sensing pathways. Cellular metabolism is under the control of nutrient-sensing pathways, which operate in response to external stimuli such as energy depletion, hypoxia and oxidative stress[55]. The two best-characterized nutrient-sensing pathways in the kidney transmit their signals via mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), respectively[56,57]. The serine/threonine kinase complex mTOR is switched on in the presence of nutrients and growth factors, and stimulates the synthesis of proteins and lipids, whereas AMPK is activated by a rise in the cellular AMP to ATP ratio and promotes catabolic processes[55,56]. mTOR enhances mitochondrial biogenesis by activating the transcriptional repressor YY1 (REFS[56,58]), which functions as a co-activator of the peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1α (PGC1α; encoded by *PPARGC1A*) to induce the expression of genes involved in mitochondrial biogenesis.

Phosphorylation of target proteins by AMPK increases glycolytic flux, fatty acid oxidation and cellular glucose uptake[59]. AMPK is rapidly activated in response to ischaemia, mainly in cortical kidney tubules[60]. AMPK promotes mitochondrial biogenesis by stimulating expression of *PPARGC1A* and by phosphorylating PGC1α to enhance its biological activity[61]. PGC1α is strongly suppressed during the acute phase of kidney injury induced by rhabdomyolysis and ischaemia in mice, but becomes fully restored during recovery from AKI[62]. Consistent with a renoprotective role of PGC1α, systemic inflammation causes kidney function to deteriorate (according to serum creatinine and blood urea nitrogen levels) more severely in *Ppargc1a*-knockout mice than in wild-type mice[63]. In addition to its role in mitochondrial biogenesis, AMPK also regulates various transport proteins along the nephron, including inhibition of the Na^+–K^+–Cl^- cotransporter (NKCC) and the epithelial Na^+ channel (ENaC), and thus has a key role in coupling tubular transport activity to the cellular metabolic state[57]. The use of these nutrient-sensing pathways as potential targets for renoprotection is discussed in detail below.

In addition to the cellular metabolic status, differences in the initiation of defence mechanisms, such as anti-inflammatory and antioxidative responses, might also contribute to the greater vulnerability of proximal tubules compared with mTAL segments under conditions that promote AKI. Specifically, cell survival in the kidney following oxidative stress depends on the balanced activation of the JUN N-terminal kinase 1 (JNK1; also known as MAPK8) and extracellular

signal-regulated kinase (ERK) pathways[64]. H_2O_2-induced necrosis of cultured mouse proximal tubule cells can be ameliorated by either stimulation of ERK or inhibition of JNK1 activity[65]. Both kinases are activated in response to ischaemia–reperfusion injury in the outer kidney medulla. By contrast, in the kidney cortex, where most of the proximal tubule is located, JNK1 activity increases but ERK activity does not, which suggests that the preponderance of pro-apoptotic signals in proximal tubular cells might contribute to their exceptional susceptibility to hypoxic injury[66].

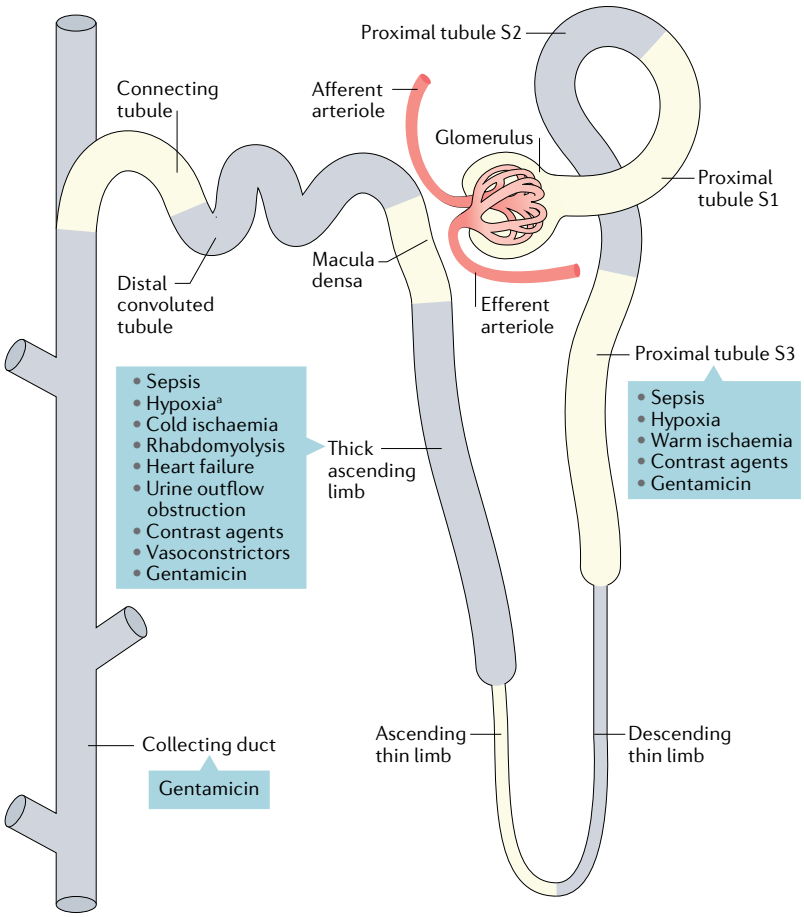


Fig. 2 | **Susceptibility of different nephron segments to various AKI aetiologies.** Differences in the local oxygen supply, cellular energy requirements, use of metabolic pathways and activation of defence mechanisms determine the variable susceptibility of different nephron segments to injury. The S_3 segments of the proximal tubules are particularly susceptible to hypoxia and warm ischaemia. Another preferential site of injury is the thick ascending limb (TAL) in the outer stripe of the kidney medulla. Hypoxic damage to the TAL segment occurs if tubular transport activity is maintained or augmented, and can be reduced by inhibition of local epithelial transporters[48]. Vulnerability of the different nephron segments is dependent on the initial damaging event. The collecting duct seems to be more resistant to most insults than the S_3 segments of the proximal tubule and the TAL but is sensitive to gentamicin-induced acute kidney injury (AKI) in mice[163]. Thus, differences in the vulnerability of the nephron segments might contribute to the heterogeneous damage patterns observed in different AKI aetiologies. Of note, this simplified scheme does not consider any combined mechanisms of kidney injury. For example, hypoxia is a critical component shared by various types of AKI, including that induced by rhabdomyolysis, contrast media and vasoconstrictors. Moreover, injury patterns are commonly studied in preclinical settings using whole animals and isolated perfused kidney preparations, and might therefore not reflect faithfully the damage patterns that are observed in human kidneys. [a]Hypoxic injury of the TAL segment occurs when the ischaemic period is prolonged or when oxygen consumption increases owing to the restoration of tubular transport during reperfusion.

Kidney damage patterns in AKI

Current AKI staging does not take into account the different underlying aetiologies and the sites of damage that can lead to kidney dysfunction. However, AKI subtypes can be identified by comparing kidney injury with variable aetiologies (FIG. 2). Available data indicate that different AKI-inducing events cause anatomically distinct patterns of injury, which are relevant for the refinement of available therapies and the development of novel renoprotective strategies. For instance, volume depletion predominantly affects the inner stripe of the outer kidney medulla[67], whereas contrast agents, rhabdomyolysis and Gram-negative sepsis impair mainly the outer stripe of the outer medulla[68–72]. The mechanisms underlying these diverse injury patterns might involve distinct cell targets in the initial damaging event, as well as heterogeneous secondary responses in the injured nephron segments.

Of note, kidney damage patterns and AKI mechanisms have been mostly studied in animal models, which might not reflect the human pathophysiology faithfully and might introduce potential biases (BOX 3). Most studies pinpoint the S_3 segment of the proximal tubule as the major site of injury in kidney ischaemia, but differences exist depending on whether kidney blood flow is interrupted while maintaining normal body temperature (warm ischaemia) or whether cold ischaemia is applied. Most animal studies use warm ischaemia to induce AKI by clamping the renal artery for a certain period of time (commonly 20–60 min). Throughout the ischaemic interval, the kidney is anoxic and non-functioning (that is, glomerular filtration and tubular transport are halted). Warm ischaemia mainly induces cellular damage in the S_3 segments, whereas the more distal nephron segments are injured when the ischaemic period is prolonged or when oxygen consumption increases owing to restoration of tubular transport during reperfusion[32,67]. By contrast, a 12-h period of cold kidney ischaemia predominantly injures the tubular TAL segments and the inner medulla in post-transplanted rats[67]. TAL damage in response to cold ischaemia might involve insufficient reflow of blood to the medulla and subsequent warm ischaemic damage of this nephron segment[73]. Notably, the proximal tubules in the outer medullary stripe and the kidney cortex are also damaged when the cold ischaemic period is extended beyond 16 h (REFS[67,73]). A pattern of predominant TAL injury is also seen in other AKI aetiologies, including heart failure, endotoxaemia, salt depletion, myoglobinuria and urine outflow obstruction[74–76]. However, the extensive TAL injury that is observed in cell-free perfused isolated kidneys is unlikely to occur in human AKI[67]. Instead, histopathological analysis of human AKI suggests that damage is usually focal and dynamic, as it depends on the timing of kidney biopsy and involves various tubular segments, mainly in the outer medullary region[67].

Tubular uptake of toxins can also cause characteristic damage patterns. For example, aminoglycoside antibiotics and the environmental pollutant cadmium are

Box 3 | **Pitfalls of the use of experimental AKI models**

- The disease mechanisms of acute kidney injury (AKI) have been mostly studied in animal models, which might not truly reflect human AKI owing to differences between species, including kidney anatomy. In particular, the common clinical setting of critical illness in patients who are on multidrug medication is difficult to recapitulate in experimental animal models.
- Warm ischaemia–reperfusion (WIR) is the most extensively applied protocol for studying AKI in animals. However, WIR is rarely a single cause of AKI in humans, and extensive damage of the S_3 segment, as observed in this model, almost never occurs in human hypoxic AKI[67].
- With the exception of kidney transplantation and cross-clamped aortic or renal artery surgery, kidney blood flow is rarely completely disrupted in clinical settings. Instead, kidney microcirculation and oxygenation are often compromised owing to, for example, systemic haemodynamic alteration, hypoxaemia and nephrotoxins.
- WIR-induced kidney injury in animals and AKI in humans differ with regard to kidney morphology and function. Tubular necrosis in human AKI is usually focal and sometimes contrasts with a marked decline in glomerular filtration rate (GFR)[166]. However, WIR in rodents causes damage mainly in the proximal tubular S_3 segments[67]. Distal nephron injury affecting the medullary thick ascending limbs might arise when distal tubule oxygen consumption exceeds the available oxygen owing to alterations in the regional microcirculation that affect oxygen supply[47].
- In particular, the extensive thick ascending limb injury observed in red blood cell-free perfused isolated kidney preparations is unlikely to occur in human AKI[67].

reabsorbed by the proximal tubules via endocytic megalin, leading to cell death in this nephron segment[77–79]. Likewise, myoglobin released from skeletal muscle upon rhabdomyolysis and filtered in the glomeruli is endocytosed by megalin and cubilin in the proximal tubules leading to the formation of reactive oxygen species (ROS) and apoptosis[80–82].

By contrast, the pathoanatomical classification of sepsis-associated AKI is less clear. Injection of rats with LPS results in endotoxin accumulation and upregulation of Toll-like receptor 4 (TLR4) in proximal tubules within 60 min, whereas distal tubules show no LPS uptake[83]. This finding suggests that certain nephron segments are more likely to respond to septic stimuli than others; yet, a pathognomonic anatomical injury pattern of sepsis-related AKI has not been elucidated. The lack of a characteristic kidney damage pattern in endotoxaemia is likely due to the complex pathophysiology of sepsis-associated AKI, which involves haemodynamic changes and metabolic reprogramming, in addition to inflammatory reactions[84].

Gene expression in AKI

RNA sequencing of distinct kidney compartments isolated through laser microdissection revealed differences in the regulation of thousands of genes in response to extracellular fluid volume contraction or kidney ischaemia. Despite a similar increase in serum creatinine levels, less than 10% of these transcripts overlapped between the two AKI models[12,70]. The majority of differentially expressed genes encode proteins involved in specific signal transduction pathways, which indicates differences in the underlying molecular mechanisms of AKI[12]. Volume depletion activated genes that are mainly involved in metabolic control (for example, gluconeogenesis and lipid metabolism) and anti-inflammatory responses, whereas kidney ischaemia stimulated a set of genes consistent with inflammatory and epithelial repair mechanisms[12,70]. Interestingly, ischaemic AKI elicits the most prominent mRNA changes in the outer stripe of the outer medulla, which, as mentioned previously, is a kidney compartment that is particularly threatened by oxygen deficiency[12]. By contrast, transcriptional changes in response to volume loss occur mainly in the inner medulla of the kidney, where final salt and water excretion are adjusted[12].

Critical disease variables such as kidney hypoxia and extracellular fluid depletion might therefore cause specific alterations in spatial gene expression during AKI. This hypothesis is also supported by the differences observed in the transcriptomic response of other AKI forms. For example, only 20% of mRNA changes overlap in rat kidneys following either kidney ischaemia–reperfusion or intraperitoneal injection of mercuric chloride, and their anatomical distribution might also vary[85]. Although most data obtained so far suggest that different aetiologies share very few genetic pathways, further studies of additional kidney injury models are needed to decipher molecular AKI subtypes comprehensively. This endeavour is being facilitated by the development of novel experimental approaches that combine transcriptomics with non-invasive imaging of specific kidney cells. This approach identified the collecting duct as a major source of ROS generation in ischaemic mouse kidneys and thereby emphasized the therapeutic potential of ROS inhibition for renoprotection[10].

Secondary events in AKI

Notably, the histopathological injury pattern not only depends on the AKI aetiology — for example, sepsis, shock and exposure to contrast media or nephrotoxins — but also on secondary events that reflect the dynamic response of kidney tissue to harmful stimuli. Notably, AKI triggering events frequently converge towards a common ischaemia–reperfusion injury pathway owing to alterations in intrarenal haemodynamics and induce kidney hypoxia[13,86,87]. The ensuing inflammation and mitochondrial damage might elicit mitophagy (that is, the removal of dysfunctional mitochondria by selective autophagy)[88] and activate the signalling cascades of regulated cell death[89]. Mitophagy is important for maintaining cellular homeostasis in the kidney and other organs by lowering ROS generation in damaged mitochondria[90]. In healthy kidneys, the rate of mitophagy is significantly higher in proximal than in distal convoluted tubules[91], which produce lower amounts of mitochondrial-derived ROS than proximal tubules[92]. Thus, variable activation of mitophagy might contribute to the heterogeneous damage patterns following kidney injury and might determine the outcome of cellular repair processes following AKI.

Renoprotective strategies

Although the structural and functional complexity of the kidney poses a major challenge in many clinical situations, it also offers a broad spectrum of potential targets for renoprotection. Here, we discuss promising therapeutic options that arise from the physiological heterogeneity of the kidney. We focus mainly on approaches that might improve tissue oxygenation and cellular energy metabolism (FIG. 3).

Targeting defective oxygen supply

As outlined above, the kidney is at constant risk of oxygen deficiency owing to its complex anatomy, heterogeneous distribution of blood and oxygen, and high energy demand (BOX 2). Moreover, kidney hypoxia is common to various types of AKI and not only predominates during the acute phase of injury but also determines the progression towards CKD. Increasing oxygen supply to the kidney and improving intrarenal oxygen distribution might therefore be an effective therapeutic approach in AKI.

Systemic hypotension and hypoxia. Defective oxygen supply to the kidney might arise from systemic causes, such as low blood pressure, or from disrupted regional oxygen delivery within the kidney[93] (TABLE 1). Although changes in kidney perfusion can affect the entire kidney, experimental models of CPB and sepsis in sheep indicate that disrupted oxygen supply to the kidney primarily affects the outer medullary region[31,33]. Current renoprotective strategies against defective systemic oxygen delivery focus primarily on re-establishing haemodynamic stability. For instance, in the treatment of systemic hypotension, the use of vasopressors such as metaraminol restores blood pressure and increases kidney perfusion to the outer medulla[30]. Similarly, when treating systemic hypoxia caused by respiratory failure, mechanical ventilation or extracorporeal membrane oxygenation is used to increase blood oxygen levels[94,95]. Studies performed in critically ill patients and in patients post-CPB reveal that increased CVP is associated with a higher incidence of AKI[96,97]. Elevated CVP causes kidney damage by decreasing kidney perfusion[98] and increasing venous congestion. Diuretics are used to prevent fluid overload, reduce CVP and re-establish oxygenation to the outer medulla. However, excessive use of diuretics might result in volume loss, low cardiac preload and low kidney blood flow. Therefore, optimal fluid management is crucial to avoid a decline in excretory kidney function.

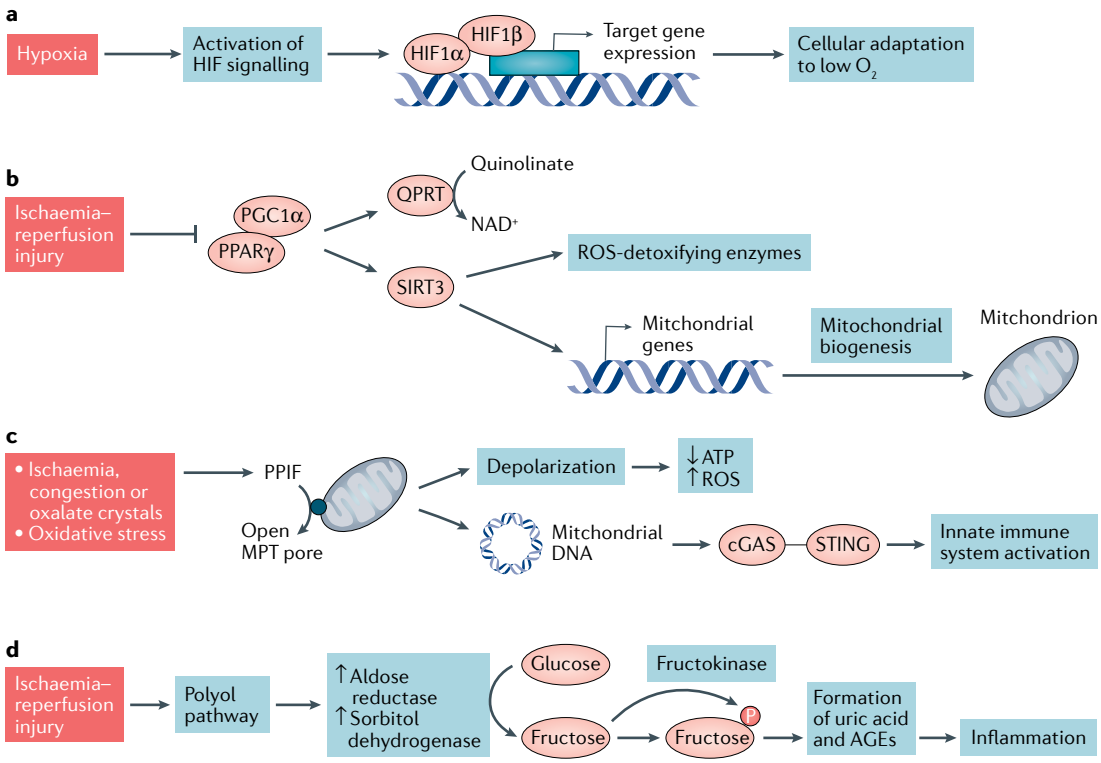


Fig. 3 | **Kidney injury pathways as potential targets for renoprotection. a** | Cellular adaptation to low partial pressure of oxygen in the kidney involves activation of hypoxia-inducible factor (HIF) target genes. Pharmacological stabilization of HIF1α is used to alleviate ischaemic kidney injury in animal models. **b** | Ischaemia–reperfusion injury inhibits mitochondrial biogenesis through a reduction in peroxisome proliferator-activated receptor-γ (PPARγ)–PPARγ co-activator 1α (PGC1α), a physiological activator of sirtuin 3 (SIRT3) and quinolinate phosphoribosyl transferase (QPRT). SIRT3 regulates reactive oxygen species (ROS)-detoxifying enzymes and mitochondrial function, whereas QPRT generates NAD^+ from quinolate. Increasing circulating NAD^+ metabolites by oral nicotinamide administration reduces acute kidney injury in patients undergoing cardiac surgery. **c** | Mitochondrial depolarization and autophagy in response to oxidative stress involves opening of the mitochondrial permeability transition (MPT) pore via the mitochondrial matrix protein peptidylprolyl isomerase F (PPIF). This opening results in ATP depletion and ROS generation. Pharmacological inhibition of the cyclic GMP–AMP synthase (cGAS) stimulator of interferon genes (STING) pathway, which activates the innate immune system in response to mitochondrial DNA leakage into the cytosol, reduced kidney injury in mice. **d** | Ischaemia–reperfusion injury stimulates the polyol pathway in the kidney through upregulation of aldose reductase and sorbitol dehydrogenase. The resulting increase in fructose leads to the production of uric acid and toxic advanced glycation end products (AGEs). Studies in patients and mice suggest that inhibition of the polyol pathway might improve excretory kidney function in acute kidney injury.

Table 1 | **Novel renoprotective strategies in AKI**

Affected processes	Pathophysiology of AKI	Targeted region	Renoprotective approach in experimental systems
Blood and/or oxygen supply to the kidney	Systemic hypotension	Entire kidney; greatest susceptibility in the outer medullary region	Use of vasopressors to increase blood pressure and kidney perfusion[30]
	Systemic hypoxia	Entire kidney; highest susceptibility in the outer medullary region	Supply of exogenous oxygen through mechanical ventilation[95] or use of extracorporeal membrane oxygenation to provide respiratory support[94]
	Elevated central venous pressure	Outer medullary region	Diuretic therapy prevents fluid overload and reduces central venous pressure, but excessive use of diuretics might result in volume loss, low cardiac preload and reduced kidney blood flow[8]
	Disrupted regional oxygen delivery within the kidney	Outer medullary descending vasa recta	In a model of cardiopulmonary bypass in sheep, increasing pump flow improved kidney blood flow and increased medullary pO_2 (REF.[30])
			Furosemide effectively increased medullary pO_2 in patients with septic shock[101]
			Combination of the α2-adrenergic agonist dexmedetomidine with a low dose of norepinephrine maintained excretory kidney function while reducing the adverse effects of a high dose of norepinephrine on the kidney medulla in a sheep model of sepsis[100]
			Hydration plus a high dose of statin with or without *N*-acetylcysteine improved protection against contrast media-induced AKI[105]
Substrate utilization and/or metabolic and oxygen-dependent signalling in kidney cells	Activation of the HIF signalling pathway	All tubular segments except the distal convoluted tubule for HIF1α targeting; vascular endothelial and tubulo-interstitial cells for HIF2α targeting	Use of the PHD inhibitor roxadustat[a] in a mouse model of cisplatin-induced AKI stabilized HIF1α and HIF2α, upregulated HIF target genes, increased glycogen storage, increased EPO levels and reduced kidney damage[132]
	Activation of the PPARγ–PGC1α pathway	Proximal and distal convoluted tubule cells; glomerular mesangial cells	Use of the NAD^+ precursors nicotinamide[139] and nicotinamide mononucleotide[140,142] increased PGC1α activity and reduced fatty acid accumulation in mouse models of ischaemia–reperfusion injury and cisplatin-induced AKI
			The AMPK activator AICAR and the antioxidant agent ALCAR induced SIRT3 activation in cisplatin-AKI, and led to a reduction in tubular injury and improved kidney function (according to BUN levels)[143]
	Mitochondrial signalling	Proximal and distal tubule cells; thick ascending limb	Ciclosporin A binds to the cytosolic protein PPIF and inhibits mitochondrial permeability transition[148]
			Inhibition of the cGAS–STING pathway with C-176 reduced kidney inflammation and fibrosis in a genetic mouse model of kidney injury[150]
	Fructokinase activation	Mainly in damaged proximal tubule cells	Treatment with luteolin, a fructokinase inhibitor, reduced kidney injury and improved kidney function determined by light microscopy and urinary excretion of NGAL in models of ischaemia–reperfusion injury and contrast-induced AKI[52]

AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AKI, acute kidney injury; ALCAR, acetyl-L-carnitine; AMPK, AMP-activated protein kinase; BUN, blood urea nitrogen; cGAS, cyclic GMP–AMP synthase; EPO, erythropoietin; HIF, hypoxia-inducible factor; NGAL, neutrophil gelatinase-associated lipocalin; PHD, prolyl-4-hydroxylase domain; pO_2, partial pressure of oxygen; PGC1α, PPARγ co-activator 1α; PPARγ, peroxisome proliferator-activated receptor-γ; PPIF, peptidylprolyl isomerase F; SIRT3, sirtuin 3; STING, stimulator of interferon genes. [a]Roxadustat, as well as the PHD inhibitors daprodustat and vadadustat, are currently being tested in clinical trials for the treatment of anaemia in patients with chronic kidney disease.

Increased intra-abdominal pressure has been found to induce AKI in rats with experimental congestive heart failure and myocardial infarction[98]. Impaired excretory kidney function in these animals was associated with haemodynamic alterations and tubular injury. Remarkably, kidney excretory function and morphology were significantly improved by pretreatment with tadalafil, which is a phosphodiesterase 5 inhibitor used to treat pulmonary hypertension and erectile dysfunction, suggesting that phosphodiesterase-5 inhibition can ameliorate the adverse effects of high intra-abdominal pressure in congestive heart failure[98].

Regional oxygen delivery within the kidney. Medullary hypoxia precedes increases in NGAL or serum creatinine by several hours[33]. Metabolic phenotyping of kidney tubule cells — including from cells obtained by routine kidney biopsy — might soon become available in the clinic using MRI-based techniques[99]. Future therapies might be aimed at improving vasa recta perfusion or limiting metabolic demand. In fact, volume expansion might offer therapeutic benefits by enhancing medullary oxygenation, as demonstrated in a sheep model of early experimental septic kidney injury[31].

Norepinephrine, a common vasopressor, is used to restore mean arterial pressure in patients with hypotension. However, renal vasoconstriction induced by high doses of norepinephrine can dramatically decrease medullary perfusion and oxygenation. A study performed in sheep revealed that using a lower dose of norepinephrine in combination with dexmedetomidine, an α2-adrenergic agonist, successfully increased creatinine

clearance while reducing the adverse effects of a high dose of norepinephrine on the medulla[100]. Similarly, a pilot study performed in patients with septic shock revealed that the diuretic furosemide, which is used to prevent fluid build-up in the body, effectively enhances medullary pO_2 (REF.[101]), perhaps by reducing oxygen demand of the TAL.

In addition to haemodynamic changes, the use of contrast media is also frequently associated with reduced medullary oxygen delivery owing to vasa recta constriction and enhanced interstitial pressure[102]. Transient increases in tubular transport and oxygen expenditure might also contribute to medullary hypoxia. Typically, minimizing the dose of contrast media and inducing volume expansion are recommended to prevent vasa recta constriction. However, in hospitalized patients, many pre-existing conditions, such as hypertension or CKD, can influence the development of AKI following contrast media administration. Therefore, the clinical relevance of AKI induced by contrast media in some patient groups, for example, in patients with sepsis, remains controversial[103], but contrast media might pose a threat for patients with an estimated GFR < 30 ml/min/1.73 m^2 (REF.[104]). A meta-analysis assessing the efficacy of various pharmacological interventions to prevent contrast media-induced AKI suggested that positive hydration plus a high dose of statin, with or without *N*-acetylcysteine, confers greater renoprotection than hydration alone[105]. The renoprotective action of statins is presumably unrelated to their lipid-lowering properties but might involve enhancement of endothelial nitric oxide production[106], antioxidative and anti-inflammatory effects[107,108], as well as prevention of apoptotic cell death[109]. Likewise, the antioxidative and vasodilatory action of *N*-acetylcysteine might be beneficial during contrast media-induced AKI[110].

Rheological measures might be considered in patients with AKI to avoid potential congestion of the sparse capillary network of the outer medulla. However, despite some promising preclinical data[111], research on such interventions has not advanced much since the initial reports and no effective therapeutic measures have been established.

Preconditioning. As treatments for AKI are often ineffective, preventing AKI by preconditioning is a potentially attractive approach. Preconditioning involves briefly exposing an organ to conditions that would induce injury during prolonged exposure so that the tissue becomes resistant to subsequent damage. For example, brief episodes of non-lethal ischaemia can protect tissues from a later ischaemic insult by activating multiple defence mechanisms, such as reprogramming of cellular energy metabolism, and stimulation of anti-apoptotic and antioxidative responses[112]. One study compared kidney gene expression signatures in a mouse model of ischaemia–reperfusion injury, in which the animals were preconditioned with either hypoxia or caloric restriction to investigate the molecular mechanisms involved in renoprotection[113]. The study demonstrated a narrow overlap in target genes between these two common preconditioning regimens, including enhanced expression of *Slc7a12*, which encodes Asc-type amino acid transporter 2, and *Aqp4*, which encodes a water channel in the basolateral membrane of principal collecting duct cells[113]. Unsurprisingly, reduced expression of genes involved in metabolism and oxidation predominated in both preconditioning forms. Gene ontology and pathway analyses indicate that, with regard to cellular components, the terms "mitochondria", "endoplasmic reticulum", "plasma membrane" and "peroxisomes" are associated with preconditioning by either hypoxia or caloric restriction. This study also identified changes in the expression of some genes that, although not previously associated with AKI, are associated with processes known to have a role in AKI, such as fatty acid metabolism (specifically, β-oxidation) or L-tryptophan metabolism[113]. β-Oxidation might be particularly relevant to the S_1 and S_2 segments because their energy supply is mainly reliant on fatty acids (FIG. 1).

Remote preconditioning refers to the induction of ischaemia–reperfusion in a distant organ. Arm cuffs are commonly used for applying ischaemia to the upper limbs and one study investigated whether applying inflation to one arm (40 mmHg above systolic pressure applied for 5 min with four repetitions) improved kidney transplant outcomes. Mortality or graft survival did not improve but a significant benefit in estimated GFR was observed[114]. Notably, in patients undergoing coronary angiography, remote preconditioning was not effective in preventing contrast media-induced AKI[115]. Moreover, remote ischaemic preconditioning in individuals exposed to bacterial endotoxins did not significantly affect levels of plasma cytokines such as TNF, IL-6 and IL-10, or levels of kidney cell-cycle arrest mediators, such as insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP2), which are markers for kidney tubule stress[116].

Hypoxic adaptation and metabolism

Profound metabolic changes and kidney hypoxia are hallmarks of different AKI aetiologies, which supports the rationale of targeting hypoxia signalling mechanisms and metabolic pathways for renoprotection. Importantly, many genes involved in metabolic control, such as *HK1* and *HK2*, which encode hexokinases that are involved in the initiation of the glycolytic pathway, are regulated by hypoxia-inducible factors (HIFs).

Activation of hypoxia-inducible factors. HIFs comprise a family of basic helix–loop–helix transcription factors that act as master regulators of gene expression in hypoxia. Three HIF isoforms have been identified — HIF1α, HIF2α and HIF3α — which share a constitutively expressed β-subunit that forms heterodimers with variable α-subunits[117]. Under normoxia, the α-subunits are hydroxylated by prolyl-4-hydroxylase domain-containing (PHD) enzymes and subsequently bind to the von Hippel–Lindau protein (pVHL)–E3-ubiquitin ligase complex for ubiquitylation and rapid proteasomal degradation. Interestingly, despite the low oxygen concentrations observed in certain regions of the kidney, HIFs are barely detectable under normal conditions. However, HIFs are robustly expressed in the

Rheological measures
Treatment protocols aimed at improving intrarenal haemodynamics and, thus, excretory kidney function.

kidney under conditions of reduced oxygen supply, such as systemic hypoxia, and AKI induced by ischaemia, nephrotoxins or sepsis[118–122]. HIF1α is detected mainly in tubular cells, whereas HIF2α is mostly expressed in vascular endothelial and peritubular cells, including in cells that produce erythropoietin[123].

The ablation of PHD2 in TAL or endothelial cells preserved kidney function by stabilizing HIF1α in a mouse model of kidney ischaemia–reperfusion[124]. Likewise, inhibition of HIF degradation with cobalt induced the expression of renoprotective genes such as *Slc2a1*, *Vegf* and *Hmox1*, and ameliorated ischaemic kidney injury in rats[125]. Thus, targeting the HIF system has therapeutic potential, and pharmacological PHD inhibitors that have been investigated since 2008 for the treatment of anaemia and other diseases might serve as effective drugs to prevent AKI. Stabilization of HIF1α and HIF2α with PHD inhibitors attenuates ischaemic kidney injury in animal models by upregulating HIF target genes and glycogen storage, and by reducing apoptosis and macrophage infiltration[126–131]. PHD inhibition also reduced kidney damage significantly in other animal models of AKI, including cisplatin-induced AKI and sepsis-associated AKI[132,133]. However, whether PHD inhibitors retain their renoprotective capacity when administered after the onset of kidney ischaemia, which would be the most common clinical need, remains controversial[134]. Several crucial parameters that determine the therapeutic efficacy of PHD inhibitors must be explored in more detail, including the timing, dosage, frequency and route (that is, oral versus parenteral) of drug administration[135]. In the past 2 years, the PHD inhibitor roxadustat was approved for the treatment of anaemia in patients with CKD in China[132,136], along with two other PHD inhibitors, daprodustat and vadadustat, in Japan[137,138]. Encouraged by this progress, HIF-targeting strategies for renoprotection should be investigated further to advance from laboratory studies to clinical application.

Activation of the PPARγ–PGC1α pathway. The transcriptional co-activator PGC1α is crucial for mitochondrial biogenesis and the regulation of cellular pathways involved in fatty acid metabolism and glucose storage, through its interaction with the nuclear receptor PPARγ[139,140]. As previously mentioned, AKI is characterized by mitochondrial dysfunction and a decrease in PPARγ–PGC1α activity in the proximal and distal convoluted tubules. Under physiological conditions, PPARγ–PGC1α regulate the expression of quinolinate phosphoribosyl transferase (QPRT; also known as nicotinate-nucleotide pyrophosphorylase (carboxylating)), the rate-limiting enzyme for de novo biosynthesis of NAD^+ from quinolinate[140]. A reduction in PPARγ–PGC1α signalling post-injury results in reduced levels of QPRT and NAD^+, which leads to mitochondrial dysfunction. Interestingly, metabolomic analysis performed in critically ill patients revealed an elevated urinary quinolinate to tryptophan ratio, which suggests that reduced QPRT might be a predictor of AKI[140]. In a phase I pilot study, administration of oral nicotinamide to patients undergoing cardiac surgery resulted in a dose-dependent increase in circulating NAD^+ metabolites and significantly reduced AKI, further supporting the hypothesis that enhanced NAD^+ biosynthesis has renoprotective effects[140].

In addition to regulating de novo NAD^+ biosynthesis, PGC1α promotes sirtuin 3 (SIRT3) activation to induce ROS-detoxifying enzymes and stimulate mitochondrial biogenesis[141,142]. In a mouse model of cisplatin-induced AKI, oxidative stress and mitochondrial damage were associated with reduced levels of SIRT3 (REF.[143]). The use of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) and the antioxidant acetyl-L-carnitine (ALCAR) induced SIRT3 activation in the same AKI model and led to reduced tubular injury and improved kidney function[143]. Furthermore, the pharmacological activation of AMPK by AICAR attenuated ischaemia-induced kidney injury, and lowered serum creatinine and urea levels in rats[144].

Targeting mitochondrial signalling. AKI increases oxidative stress, which leads to mitochondrial depolarization and autophagy. The breakdown of the mitochondrial membrane increases water influx and promotes mitochondrial swelling[145]. Peptidylprolyl isomerase F (PPIF) is a mitochondrial matrix protein that regulates the opening of the mitochondrial permeability transition (MPT) pore. When the pore is open, the inner mitochondrial membrane is depolarized, which leads to a decrease in ATP formation and increased ROS production[146]. Ischaemia[147], congestion[68] and oxalate crystal formation[148] have all been shown to activate PPIF-dependent MPT, thereby increasing ROS production. Interestingly, studies performed in a model of oxalate-induced AKI in mice, revealed that the use of ciclosporin A, a PPIF inhibitor, is sufficient to inhibit the opening of the MPT pore and suppress crystal-induced cell death in tubule epithelial cells[148]. Further research is required to determine whether this approach might be beneficial in other models of AKI. Importantly, any therapeutic use of ciclosporin A for the treatment of AKI would have to take into account the potential adverse effect of this compound, which includes a risk of causing excretory kidney function to deteriorate[149].

Mitochondrial damage has been reported to cause inflammation and promote the progression of AKI to kidney fibrosis. The cyclic GMP–AMP synthase (cGAS) stimulator of interferon genes (STING) pathway has a crucial role in this process[150,151]. STING is an endoplasmic reticulum protein that induces the production of type I interferons in response to cytosolic DNA and other pathogen-associated molecular patterns[152,153]. cGAS functions as a cytosolic sensor of pathogen-derived DNA and stimulates STING[154], which leads to activation of the innate immune system. Remarkably, the cGAS–STING pathway is activated in a cisplatin-induced mouse model of AKI owing to leakage of mitochondrial DNA into the cytosol[151]. The ensuing inflammation and disease progression are reduced in STING-deficient mice[151]. Furthermore, kidney tubule-specific deletion of mitochondrial transcription factor A (TFAM) in mice caused severe mitochondrial loss, kidney fibrosis and inflammation, but these pathological changes

were ameliorated by genetic deletion or pharmacological inhibition of STING[150]. These data identify the cGAS–STING pathway as a novel candidate target for preventing the progression from AKI to CKD.

Fructokinase inhibition. As previously mentioned, AKI induces the activation of the polyol pathway, which leads to the formation of uric acid and toxic advanced glycation end products[52,53]. Remarkably, in mouse models of contrast media-induced AKI or ischaemia–reperfusion, treatment with the fructokinase inhibitor luteolin reduced kidney injury, as determined by light microscopy and urinary excretion of NGAL, and improved excretory kidney function according to serum creatinine concentration[52]. Given that patients with AKI have elevated levels of urinary fructose[52], inhibition of fructokinase, potentially through the use of luteolin, might limit kidney injury and prevent a decline in kidney function in patients.

Conclusions

AKI defined by a rapid decline in GFR and/or urine flow is a multifaceted clinical syndrome with numerous underlying aetiologies. Activation of gene sets with little overlap in different anatomical regions of the kidney suggests that distinct molecular AKI subtypes exist[12,13,70,85]. These subtypes appear to correspond, at least to some extent, to distinct AKI aetiologies, suggesting that the use of biomarkers might in future enable the identification of the cause or causes of AKI and enable more specific preventive or therapeutic interventions[155]. Whether different types of kidney injury converge into a common final pathway remains unclear. Of note, fibrosis, which is a hallmark of CKD, might occur early in the AKI–CKD transition and might represent a more universal therapeutic target for renoprotection[9,156,157].

Against this background, we propose that future renoprotective strategies must take greater account of the complexity of AKI and address a broad spectrum of disease mechanisms. As such, successful approaches might build upon the improvement of kidney tissue oxygenation, activation of cellular defence mechanisms, including anti-apoptotic, antioxidative and anti-inflammatory reactions, and modulation of cellular energy metabolism (that is, mitochondrial function). Reduced kidney oxygen supply is a critical factor shared by many AKI aetiologies. HIFs are master regulators of adaptational gene expression during oxygen deficiency that have an important role in kidney injury and repair. Accordingly, the use of new compounds that activate the HIF pathway in normoxia shows early promise in preclinical AKI models[117,126,128–130,132,135]. The discovery of the contribution of gut microbiota-derived metabolites to renocardiovascular health is another promising area of research[158–160]. Short-chain fatty acids from gut microbiota are anti-inflammatory and prevent ischaemia–reperfusion-induced AKI in mice, presumably by enhancing mitochondrial biogenesis[158]. The main microbial products in the gut are D-amino acids, of which D-serine is the only molecule detectable in normal mouse kidney[161]. Remarkably, the D-serine to L-serine ratio was increased in the kidneys of mice with ischaemia–reperfusion injury compared with sham-operated mice, and oral administration of D-serine attenuated hypoxia-induced tubular damage in this AKI model[161]. The plasma D-serine to L-serine ratio also correlated positively with serum creatinine concentrations in patients with AKI[161].

Early evidence suggests that the specific structural and functional organization of the kidney, in combination with the complexity of AKI aetiologies — two factors that have long impeded the development of therapies for AKI — might eventually enable targeted approaches. The translation of advances in transcriptome analysis and metabolomics, in particular at single-cell resolution, into clinical application is eagerly awaited.

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Competing interests
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