Studying the short-term complications of kidney transplantation: from bed to bench

Kok, M.J.C. de

Citation

Kok, M. J. C. de. (2022, October 11). *Studying the short-term complications of kidney transplantation: from bed to bench*. Retrieved from https://hdl.handle.net/1887/3479720

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/3479720

Note: To cite this publication please use the final published version (if applicable).

Chapter 6

Preclinical models versus clinical renal ischemia reperfusion injury: a systematic review based on metabolic signatures.

Lente J.S. Lerink*, Michèle J.C. de Kok*, John F. Mulvey, Sylvia E. Le Dévédec, Alexander A. Markovski, Rob C.I. Wüst, Ian P.J. Alwayn, Rutger J. Ploeg, Alexander F.M. Schaapherder, Jaap A. Bakker, Jan H.N. Lindeman
* Both authors contributed equally to this work

American Journal of Transplantation. 2021;00:1-27.

Abstract

Despite decennia of research and numerous successful interventions in the preclinical setting, renal ischemia reperfusion (I/R) injury remains a major problem in clinical practice, pointing towards a translational gap. Recently, two clinical studies on renal I/R injury (manifested either as acute kidney injury or as delayed graft function), identified metabolic derailment as a key driver of renal I/R injury. It was reasoned that these unambiguous metabolic findings enable direct alignment of clinical with preclinical data, thereby providing the opportunity to elaborate potential translational hurdles between preclinical research and the clinical context. A systematic review of studies that reported metabolic data in the context of renal IR was performed according to the PRISMA guidelines. The search (December 2020) identified thirty-five heterogeneous preclinical studies. The applied methodologies were compared, and metabolic outcomes were semi-quantified and aligned with the clinical data. This review identifies profound methodological challenges, such as the definition of I/R injury, the follow-up time and sampling techniques, as well as shortcomings in the reported metabolic information. In light of these findings, recommendations are provided in order to improve the translatability of preclinical models of renal I/R injury.

Introduction

Ischemia reperfusion (IR) injury describes the paradoxical increase in tissue injury following reperfusion of transiently ischemic organs. I/R injury contributes significantly to graft damage in the context of organ transplantation. Unfortunately, despite decades of research and numerous preclinical successes, no intervention to date successfully reduced clinical I/R injury.[1,2]

The notable contrast between preclinical successes and consistent clinical failures points towards a profound translational gap in the understanding of I/R injury. Independently of each other, two recent clinical studies implied metabolic failure as the primary effector mechanism of renal I/R injury. To be specific, these studies concluded that both delayed graft function (DGF) in the context of kidney transplantation, as well as acute kidney injury (AKI) in the context of major cardiac surgery, associate with profound, transient post-reperfusion metabolic defects such as, in the case of DGF, post-reperfusion normoxic glycolysis and persistent post-reperfusion ATP catabolism (further details are summarized in Table 1 and Figure 1).[3,4]

We reasoned that these unambiguous observations for clinical renal I/R injury provide the opportunity to validate reported preclinical models. Therefore, we performed a systematic literature review to identify studies that report on metabolic aspects of experimental renal I/R injury. Methodological aspects and reported metabolic observations in these studies were aligned with the clinical context in an attempt to map parallels and dissimilarities between preclinical models and clinical context.

Table 1. Two recent clinical studies reporting renal metabolic data after ischemia and reperfusion (IR) resulting in acute kidney injury (AKI) or delayed graft function (DGF)[3,4].

Article	Sample timing	IR injury definition	Results on metabolome
Legouis et al. 2020[3]	Blood: twice at a 30-min interval. Control group: 4-6 h post-IR; AKI group: 2-6 days post-IR.	AKI: KDIGO criteria	In patients experiencing AKI: switch from net renal lactate uptake to net renal lactate release, a decrease in net renal glucose release compared to that in the control group.
Lindeman et al. 2020[4]	Blood: renal artery: 0, 10, 30 min post-IR; renal vein: 0.5, 3, 5, 10, 20, 30 min post-IR Tissue: post-ischemia and 45 min post-IR	DGF: recipient requires dialysis in first week(s) post-transplantation, excluding dialysis for hypervolemia, hyperkalemia or hyperphosphatemia.	Grafts manifesting future DGF: post-reperfusion ATP/GTP catabolism (significantly impaired phosphocreatine recovery and significant persistent (hypo)xanthine production). Failing high-energy phosphate recovery occurred despite activated glycolysis, fatty-acid oxidation, glutaminolysis and autophagia, and related to a defect at the level of the oxoglutarate dehydrogenase complex in the Krebs cycle.

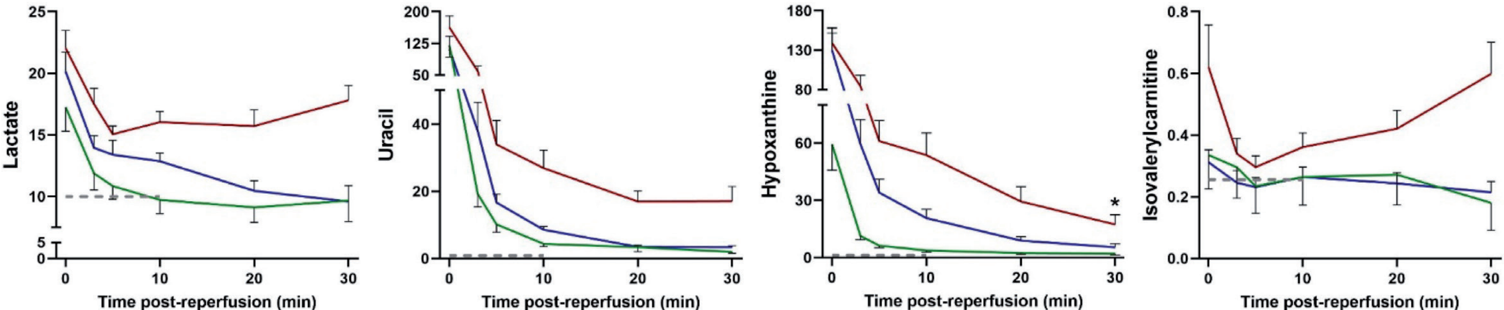


Figure 1. Illustration of the contrasting post-reperfusion metabolic responses of kidney donor grafts with Delayed Graft Function (DGF, IR injury, red curve) and grafts recovering without IR injury (no DGF) (green curve: living donor graft (intermediate ischemic period), blue curve: deceased donor graft (prolonged ischemic period)). The dashed line reflects the normal, non-ischemic kidney. Figures adapted from Lindeman et al.[4]. Curves represent renal vein levels of lactate (glycolysis), uracil (cellular damage)[5], hypoxanthine (ATP catabolism, metabolic incompetence), and isovalerylcarnitine (intermediate of branched chain amino acid oxidation (autophagia)). *: decrease at end of measurement window may reflect depletion of ATP pool.

Methods

Systematic searches (see Supporting Information) were performed in PubMed, EMBASE and Web of Science to identify preclinical studies reporting metabolic data following renal IR in the context of DGF and AKI. Articles were selected following recommended procedures described by PRISMA guidelines.[6] Two authors independently assessed titles and abstracts for eligibility. Full texts were consulted if it was unclear whether inclusion criteria were met.

Results

The literature searches are summarized in two diagrams (Supporting Figure 1). Thirty-four studies were selected based on the predefined inclusion criteria. One extra study (unidentified in both searches) was included. Thus, thirty-five preclinical studies were included, of which seventeen explored renal IR in rats, ten in mice, one in both rats and mice (included as two separate studies), four in pigs, and two in dogs. Almost all studies were performed in homogeneous populations of particularly young, mostly male, and healthy animals. The dog studies included an explicitly heterogeneous study population of mongrel dogs. Details of the methodology and results of the included reports are summarized in Table 2 and 3.

Measures of renal I/R injury

Although some variability exists for the clinical definitions of AKI and DGF (Table 4), definitions are essentially functional and outcome-centered.[40,41] The two landmark clinical studies that report the metabolome of respectively AKI and DGF both used 'conservative' definitions.[3,4] The diagnosis of AKI was based on KDIGO criteria (Table 4)[41], and DGF was defined as the need for dialysis for at least the first week after transplantation.[40] Partial or full functional recovery is an inherent aspect of both clinical definitions.
The majority (23/35) of the included preclinical studies used post-reperfusion serum creatinine levels as a functional measure of I/R injury (Table 3). In contrast to clinical definitions of AKI, no predefined thresholds for the diagnosis of I/R injury were considered. None of the preclinical studies included the need for (transient) renal replacement therapy as outcome measure. A third of the studies (11/35) reported histological grading as surrogate outcome parameter (Table 3). Other parameters used were diverse: e.g. body and/or kidney weight, and expression of the damage markers kidney injury molecule-1 (KIM-1) and/or neutrophil gelatinase-associated lipocalin (NGAL).
The dynamics of recovery is an inherent aspect of the clinical diagnosis of renal I/R injury (Table 4), but could not be properly addressed in the majority of experimental studies

Table 2. Methodological details reported in the publications included by the systematic search.

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Rat										
Andrianova et al. 2020[7]	Rat	OutbredWistar rats Male	3-4 months 300-400 g	Clamping Right nephrectomy, clamping of left renal vascular bundle for 40 min.	-	40	Blood (carotid artery)	Blood: 48 h post-IR	Blood: intact control	Blood metabolomics: FIA-MS/MS Serum levels urea & creatinine: AU480 Chemistry System
Choi et al. 2019[8]	Rat	Sprague-Dawley rats Male	3-4 months 410-450 g	Surgery 20 min of cardiac arrest through asphyxia, then resuscitation by cardiopulmonary bypass.	-	20	Tissue	Tissue: post-I and 30 min post-IR After 30 minutes of cardiopulmonary bypass resuscitation, rats were euthanized to harvest brain, heart, kidney, and liver tissue samples.	Tissue: intact control, no cardiac arrest, euthanized 7 min before harvesting tissues.	Tissue metabolomics: LC-MS/MS
Duran et al. 1990[9]	Rat	Sprague-Dawley rats Female	??? 240-312 g	Clamping Unilateral clamping of left renal artery for 1 h.	-	60	Blood (cardiac puncture) Tissue (cortex)	Blood, tissue: 3 and 24 h post-IR	Blood, tissue: “control” rats Blood (for BUN): prior to IR, from tail	Blood/tissue metabolomics: dansylation of amino acids and subsequent chromatography BUN: autoanalyzer technique
Gaudio et al. 1991[10]	Rat	Sprague-Dawley rats	??? 250 g	Clamping Clamping of aorta proximal to both renal arteries for 45 min.	-	45	Tissue	Tissue: 15 min or 2 h post-IR, measurements om proximal tubule suspensions.	Tissue: sham-operated rats	ATP content: HPLC
Huang et al. 201[81]1	Rat	Fisher F344 rats	??? 250-300 g	Clamping Unilateral clamping of left renal artery for 45 min.	-	45	Blood (???) Tissue (cortex)	Blood, tissue: 4 h and 24 h post-IR	Blood and tissue: contralateral kidney and kidneys from healthy control rats	Tissue metabolomics: ^{1}H-NMR & GCxGC-MS
Lan et al. 2016[12]	Rat	Sprague-Dawley rats Male	???	Clamping Right nephrectomy, clamping of left renal vascular pedicle for 45 min.	-	45	Blood (???) Tissue (cortex and outer stripe of outer medulla)	Blood: daily post-IR Tissue: 3, 7 and 14 days post-IR	Blood: prior to surgery Tissue: sham-operated rats	Tissue lactate and pyruvate levels: fluorimetry SCr: ???

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Legouis et al. 2020[3]	Rat	Sprague–Dawley rats Male	8-10 weeks ???	Clamping Right nephrectomy, clamping of left renal artery for 25 min.	-	25	Blood (left femoral artery, left femoral vein and renal vein)	Blood: 60 and 120 min post-IR	Blood: sham-operated rats	Gluconeogenesis (plasma D2-glucose enrichment after administration of D2-glucose) measurement in blood: GC-MS
Lindhardt et al. 2020[13]	Rat	Wistar rats Male	??? 205-290 g	Clamping Unilateral clamping of left renal artery for 40 min.	-	40	Blood (tail vein) Tissue imaging *in vivo* Urine (metabolic cage)	Blood, tissue (imaging *in vivo*): 24 h post-IR Urine: 24 h post-IR	Blood: no control Tissue imaging *in vivo*: contralateral kidney Urine: no control	Tissue metabolomics: *In vivo* ^{1}H and hyperpolarized ^{13}C MRI Urinary creatinine, BUN and SCr: COBAS 6000 device (Roche).
Liu et al. 2012[14]	Rat	Srague-Dawley rats Male	Adult 175-225 g	Clamping Clamping of renal arteries for 45 min.	-	45	Blood (posterior orbital venous plexus) Tissue (cortex)	Blood: 2, 4, 6, 12, 24, 48, 72 and 96 h post-IR Tissue: 24 h and 96 h post-IR	Blood and tissue: sham-operated rats	Blood metabolomics: HPLC/MS
Nielsen et al. 2017a[15]	Rat	Wistar rats Male	??? 200-250 g	Clamping Unilateral clamping of left renal artery for 40 min.	-	40	Blood (arterial) Tissue Urine (metabolic cage)	Blood, tissue imaging *in vivo* and samples: 24 h post-IR Urine: 24 h post-IR	Blood: prior to surgery Tissue (imaging *in vivo* and samples): contralateral kidney Urine: 24 h prior to surgery	Tissue metabolomics: *In vivo* ^{1}H and hyperpolarized ^{13}C MRI
Nielsen et al. 2017b[16]	Rat	Wistar rats Male	??? 250-290 g	Clamping Unilateral clamping of left renal artery for 30 or 60 min.	-	30 or 60	Blood (??? before surgery and aorta post-IR) Tissue imaging *in vivo* Tissue samples (cortex)	Blood, tissue imaging *in vivo* and samples: 24 h post-IR	Blood: prior to surgery (24 h) and sham-operated rats Tissue: contralateral kidneys and sham-operated rats	Tissue metabolomics: *In vivo* ^{1}H and hyperpolarized ^{13}C MRI Tissue lactate levels: enzymatic assay BUN and SCr: COBAS 6000 device (Roche) Renal KIM-1 and NGAL expression: qPCR

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Nielsen et al. 2020[17]	Rat	Wistar rats Male	??? 200-245 g	Clamping (2 distinct procedures) Procedure 1: Unilateral clamping of left renal artery for 30 min. Procedure 2: Unilateral clamping of left renal artery for 20 or 40 min.	-	20, 30 or 40	Blood (teil vein) Tissue imaging *in vivo* Tissue samples (cortex and inner medulla)	Blood: directly before tissue imaging *in vivo* Tissue imaging *in vivo*: Procedure 1 – 2 and 60 min post-IR Procedure 2 – 1 and 7 days post-IR Tissue samples: 60 min post-IR and 7 days post-IR	Blood: no control Tissue (imaging *in vivo* and samples): contralateral kidney	Tissue metabolomics: *In vivo* ^{1}H and hyperpolarized ^{13}C MRI SCr: COBAS 6000 device (Roche)
Peto et al. 2018[18]	Rat	Crl:WI rats Male	??? 342.2 ± 29.5 g	Clamping Ligation of right renal artery and clamping of left renal vessels for 60 min. After WI, excision of right kidney and clamp removal from the left renal vessels.	-	60	Blood (left femoral artery) Tissue	Tissue: 120 min post-IR Blood: pre-I, post-I, 60 min post-IR and 120 min post-IR	Blood and tissue: prior to surgery and sham-operated rats	Blood acid-base parameters, glucose and electrolytes: EPOC portable blood analysis device
Serkova et al. 2005[19]	Rat	Lewis rats Male	??? 200-250 g	Transplantation After removal from living donors, kidneys were kept cold for 24 or 42 h. Implantation after removal of both kidneys from recipient.	24 or 42	???	Blood (???) Tissue	Blood: pre-IR, 24 h post-IR Tissue: pre-IR, post-I, 24 h post-IR	Blood and tissue: recipient's kidney and blood prior to nephrectomy and transplantation	Blood/tissue metabolomics: ^{1}H-NMR
Shen et al. 2017[20]	Rat	Sprague-Dawley rats Female	Adult 200-220 g	Clamping Clamping of renal pedicles for 30 min.	-	30	Tissue	Tissue: post-IR (reperfusion time unknown)	Tissue: sham-operated rats	Tissue metabolomics: GC/MS
Tani et al. 2019[21]	Rat	Sprague-Dawley rats Male	6 weeks ???	Clamping Unilateral clamping of left renal pedicle for 30 min.	-	30	Tissue	Tissue: 60 min after drug administration, post-I, and 30 min post-IR	Tissue: "vehicle treatment group", no surgery, 60 min after receiving 0.5 mL 0.5% methylcellulose.	Tissue purine nucleotide concentration: HPLC Tissue metabolomics: CE-ToFMS

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Trifillis et al. 1984[22]	Rat	Sprague-Dawley rats Male	??? 220-250 g	Clamping Clamping of aorta above left renal artery, below the right renal artery and superior mesenteric artery, as well as clamping right renal artery and vein for 15, 10, 60, 90 or 120 min.	-	5, 15, 30, 60, 90 or 120	Blood (aorta) Tissue	Blood: ??? Tissue: post-I, and 0.25, 1, 6, 24 or 48 h post-IR	Blood: ??? Tissue: control rats (no sham surgery)	Tissue ATP/ADP/AMP/lactate measurements: specific enzymatic methods coupled with NADH or NADPH Tissue Pi measurements: modification of Fiske and SubbaRow method SCr: Beckman creatinine analyzer II. BUN: Beckman urea nitrogen analyzer.
Varga et al. 2019[23]	Rat	Crl:WI rats Male	??? 301.6 ± 38.6 g	Clamping Unilateral clamping of left renal vessels for 45 min.	-	45	Blood (right femoral artery)	Blood: pre-I, and 30, 60 and 120 min post-IR	Blood: prior to surgery and sham-operated rats	Blood acid-base parameters, metabolites and electrolytes: EPOC portable blood analysis device
Mouse										
Beier et al. 2020[24]	Mouse	C57BL/6 mice Female	???	Clamping Unilateral clamping of renal pedicle for 28 min.		28	Tissue	Tissue: 24 h post-IR	Tissue: contralateral kidney	Tissue metabolomics: UPLC-MS
Chihanga et al. 201[82]5	Mouse	Swiss-Webster mice	??? 25-30g	Clamping Clamping of renal pedicles for 30 min.	-	30	Blood (inferior vena cava for SCr, cardiac puncture for NMR) Tissue Urine (metabolic cage)	Blood: 24 h post-IR Tissue: 24 h post-IR Urine: 3 days pre-IR and 24 h post-IR	Blood: control mice (pre-IR) Tissue: control mice (pre-IR) Urine: 3 days pre-IR	Blood/urine metabolomics: ^{1}H-NMR Urinary creatinine, SCr and urinary NGAL: spectroscopy

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Cho et al. 2017[26]	Mouse	C57BL/6 mice Male	9 weeks ???	Clamping Unilateral clamping of left renal pedicle for 45 min.	-	45	Blood (???) Tissue Urine (metabolic cage)	Blood: 24 h post-IR Tissue: 24 h post-IR Urine: 24 h post-IR	Blood, tissue and urine: sham-operated mice	Blood/tissue/urine metabolomics: HPLC-Q-ToF MS
*Chouchani et al. 2014[27]	Mouse	C57BL/6J mice Male	8-10 weeks ???	Clamping Unilateral clamping of one renal pedicle for 45 min.	-	45	Tissue	Tissue: post-I and 5 min post-IR	Tissue: ??? (normoxic controls)	Tissue metabolomics: LC-MS
Fujii et al. 201[92]8	Mouse	C57BL/6 Male	8 weeks ???	Clamping Unilateral clamping of left renal artery for 1, 10 and 40 min.	-	1, 10 and 40	Blood (???) Tissue Urine (???)	Tissue: after 1, 10, and 40 min WI, and 24 h post-IR Blood and urine: 24 h post-IR	Blood and urine: ??? Tissue: sham-operated mice	Tissue metabolomics: Matrix-assisted laser desorption/ ionization–imaging mass spectrometry (MALDI-IMS) + data calibration by CE-MS. Serum/urine samples: "standard method"
Jouret et al. 2016[29]	Mouse	C57BL/6J mice Male	10 weeks ~20 g	Clamping Clamping of renal pedicles for 30 min.	-	30	Blood (vena cava) Tissue Urine (metabolic cage)	Blood, tissue, and urine: 6 h, 24 h and 48 h post-IR	Blood, tissue and urine: sham-operated mice	Blood/tissue/urine metabolomics: ^{1}H-NMR Serum levels urea & creatinine: COBAS 6000 device (Roche).
Legouis et al. 2020[3]	Mouse	C57Bl/6 J mice Male	10-12 weeks 25–28-g	Clamping Clamping of renal pedicles for 25 min.	-	25	Blood (???) Urine (???)	Blood and urine: 24 and 48 h post-IR Blood (for lactate clearance test): 15, 30, 60, 90, 120 min post-injection of sodium lactate (injection at 6 h post-IR)	Blood and urine: sham-operated mice	Blood lactate and glucose: Aviva Accu-Check glucometer and Novabio StatStrip Xpress lactate meter. SCr: capillary electrophoresis BUN: quantitative colorimetric determination using Stanbio Excel analyser

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Poyan Mehr et al. 2018[30]	Mouse	C57BL/6J mice Male	8-12 weeks ???	Clamping Clamping of renal pedicles for 20 min.	-	25	Blood (???) Tissue Urine (???)	Blood, urine and tissue: 24 h post-IR	Blood, tissue and urine: sham-operated mice	Tissue/urine metabolomics: LC-MS SCr: LC-MS
Rao et al. 2016[31]	Mouse	C57BL/6 mice Male	10-12 weeks ???	Clamping Right nephrectomy, clamping of left renal pedicle for 30 min.	-	30	Tissue	Tissue: 6 and 24 h post-IR	Tissue: sham-operated mice	Tissue lipid concentrations: SWATH-MS Tissue hydroxyoctadeca dienoic acid/ hydroxyeicosatetra enoic acid measurement: LC-MS/MS
Wei et al. 2014[32]	Mouse	C57BL/6 mice Male	9 weeks ???	Clamping Clamping of renal pedicles for 25 min.	-	25	Blood (???) Tissue (cortex and medulla)	Blood and tissue: 2 h, 48 h or 1 week post-IR	Blood and tissue: sham-operated mice	Blood/tissue metabolomics: GC/MS and LC/MS
Zager et al. 2014[33]	Mouse	CD-1 mice Male	??? 30-45 g	Clamping Unilateral clamping of left renal pedicle for 15, 30 or 60 min.	-	15, 30 or 60	Blood (vena cava) Tissue (cortex)	Blood and tissue: after 15, 30, or 60 min WI, and 2 or 18 h post-IR	Blood: sham-operated mice Tissue: contralateral kidney and sham-operated mice	Tissue/blood lactate, pyruvate, glucose and glycogen levels: enzymatic assays
Pig										
Clendenen et al. 2019[34]	Pig	Farm pigs Male	??? 50-55 kg	Clamping Clamping of renal pedicles for 30 min.	-	30	Blood (renal vein)	Blood: pre-IR, after 15 and 30 min WI, and 5 min post-IR	Blood: pre-IR	Blood metabolomics: UHPLC-MS

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Fonouni et al. 2011[35]	Pig	Landrace pigs	??? 26-33 kg	Transplantation Living donor left kidney explantation (30 min), implantation in recipient after 6 h CI. 120 min reperfusion.	6	60 (anastomosis)	Blood (???) Extracellular fluid (microdialysis = MD)	Blood: during procurement, post-I, and 120 min post-IR MD: 10-min intervals during kidney procurement, CI (2 samples in the first and 2 samples at the end of CI), and at 20-min intervals during kidney implantation (WI) and post-reperfusion (120 min).	Blood and extracellular fluid: during explantation procedure	MD: CMA 600 Microdialysis Analyzer Blood analysis: hospital laboratory
Hauet et al. 2000[36]	Pig	Large White pigs Male	??? 41–52 kg	Transplantation Left nephrectomy, kidneys were flushed with EC solution or UW solution. After 48 h CI, heterotopic auto-transplantation was performed and contralateral nephrectomy was carried out.	48	???	Blood (right jugular vein) Urine (metabolic cage)	Blood and urine: two days before kidney preservation (D-2) and at 1, 3, 5, 7, 11, and 14 days (D1–D14) post-IR. Also examined these kidneys 30–40 min after implantation and on the sacrifice day.	Blood and urine: control group uninephrectomized, no flushing or cold preservation	Blood/urine metabolomics: ^{1}H-NMR
Malagrino et al. 2019[37]	Pig	Pigs Female	Juvenile 15-20 kg	Clamping Unilateral clamping of the right renal artery for 120 min.	-	120	Blood (inferior vena cava above the renal veins) Tissue Urine (directly from bladder)	Tissue: 24 h post-IR Blood, urine: pre-IR (before occlusion), after 1 h WI, 0.5 h post-IR, 4 or 6 h post-IR, and 11 h post-IR	Tissue: contralateral kidney Blood and urine: pre-IR samples	Blood/urine metabolomics: ^{1}H-NMR

Article	Species	Breed	Age Weight	Ischemia: transplantation, surgery or clamping	Cold ischemia time (h)	Warm ischemia time (min)	Sample type	Sampling time points	Control(s)	Relevant measurement techniques
Dog										
Maessen et al. 198[93]8	Dog	Mongrel dogs	Adult 18-25 kg	Transplantation Clamping of left renal vessel pedicle for 0 or 30 min. Then kidney explantation and storage on ice. Autologous reimplantation, contralateral nephrectomy.	24 or 48	0 or 30 + ???	Tissue (cortex)	Tissue: after WI, after CI, and 1 h post-IR	Tissue: non-ischemic control	Tissue energy metabolite levels: HPLC
Montanés et al. 1991[39]	Dog	Mongrel dogs	??? 17-34 kg	Clamping Right nephrectomy, clamping of left renal artery for 60 min.	-	60	Blood (femoral artery, left ovarian, or spermatic vein) Tissue (cortex) Urine (left ureter and metabolic cage)	Blood, tissue, and urine: 2 days post-IR	Blood, tissue and urine: sham-operated dogs	Blood/tissue metabolomics: enzymatic assays Scr: enzymatic assay Blood/urine pH and gasses: blood gas analyzer model 168 (Corning Medical)

*For the sake of clarity, the induction of ischemia was classified as "clamping", including several approaches to occlude blood flow, or "transplantation", in which the kidney was removed and transplanted. AV: arterio-venous. BUN: blood urea nitrogen. CE-MS: capillary electrophoresis-mass spectrometry. CE-ToFMS: capillary electrophoresis–time of flight mass spectrometry. CI: cold ischemia. DGF: delayed graft function. fDGF: functional DGF. FIA-MS/MS: flow injection analysis tandem mass spectrometry. GC: gas chromatography. GCxGC-MS: 2-dimensional gas chromatography-mass spectrometry. HPLC-Q-ToF MS: high-performance liquid chromatography–quadrupole-time of flight mass spectrometry. IR: ischemia reperfusion. KIM-1: kidney injury molecule-1. LC: liquid chromatography. MALDI-MS: matrix-assisted laser desorption/ionization mass spectrometry. MD: microdialysis. MRI: magnetic resonance imaging. MRS: magnetic resonance spectroscopy. NGAL: neutrophil gelatinase-associated lipocalin. NMR: nuclear magnetic resonance. Post-I: post-ischemia. Pre-I: pre-ischemia. qPCR: quantitative polymerase chain reaction. SCr: serum creatinine. SWATH-MS: sequential window acquisition of theoretical spectra-mass spectrometry. UHPLC: ultra-high performance liquid chromatography. UPLC-MS/MS: ultra-performance liquid chromatography tandem mass spectrometry. WI: warm ischemia. ???: data unknown. *: additionally included study.*

Table 3. Results reported in the publications included by the systematic search.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Rat					
Andrianova et al. 2020[7]	Rat	Serum creatinine (SCr) levels: "After IR, creatinine concentration increased in all animals, varying from 50 to 200–500 µM, indicating that IR caused AKI" *Note: no thresholds given.*	SCr (increase post-IR)	-	Only measured acylcarnitines (ACs) and amino acids (AAs). After IR, we detected 34 metabolites in blood serum, whose levels significantly changed – concentration of 31 acylcarnitines increased, while the content of 3 AAs (tyrosine, tryptophan, and proline) dropped. The most significant changes were observed for malonylcarnitine, which demonstrated a 7-fold increase compared to control, glutarylcarnitine (5-fold increase), decadienoyl.arnitine (4-fold increase), hydroxybutyrylcarnitine (4-fold increase), linoleylcarnitine (4-fold increase), and methylmalonylcarnitine (4-fold increase). Other acylcarnitines showed about a 2-fold increase. The serum levels of the tyrosine, tryptophan, and proline concentration dropped to 60%–70% of their content
Choi et al. 2019[8]	Rat	Not defined	-	-	Principal component analysis: Significant separation following cardiac arrest (CA) and following resuscitation. Citrate, a-ketoglutarate, malate, fumarate, and succinate significantly changed in kidney after cardiopulmonary bypass (CPB) compared with control or CA. Individual metabolites changes: -The only amino acids that were unaffected by CA were glutamine, threonine, alanine, and valine, remaining measured amino acids were significantly decreased. 30 minutes of CPB resuscitation resulted in even more dysregulation; all measured amino acids with the exception of glutamate, alanine, and valine were significantly decreased. -30 minutes of CPB resuscitation resulted in a significant decrease in linoleic and linolenic acids, while stearic acid returned to control levels. The general trend of lipids in the kidney is that they increase after ischemia, but either return to or fall below control levels after resuscitation. -The kidney shows variations in glycolytic and TCA cycle metabolites, such as a significant elevation in 3-phosphoglycerate and oxoglutarate after 20 minutes of CA. However, CPB resuscitation resulted in a significant increase in citrate, oxoglutarate, succinate, fumarate, malate, and oxaloacetate. -The urea cycle metabolites were mostly altered after 30 minutes of CPB resuscitation in the kidney. -Arginine and proline were significantly decreased after CA, but after CPB, arginine, citrulline, ornithine, and proline were all decreased. Changes in acylcarnitine species: CA resulted in elevation in only AC 16:0 when compared with control. Resuscitation resulted in a decrease from CA in AC 2:0, AC 8:0, AC 10:0, AC 14:2, AC 16:1, AC 16:0, AC 18:2, and AC 18:1. AC species that were decreased after resuscitation were AC 6:0, AC 8:0, AC 10:0, AC 14:2, AC 14:1, AC 16:2, AC 16:1, AC 18:2, and AC 18:1. The kidney was unable to normalize ACs after resuscitation, resulting in a generally diminished lipid reserve after 30 minutes of CPB resuscitation. Pathway analysis: In kidney tissue following resuscitation, 46 pathways remained significantly altered compared with control, with nicotinate and nicotinamide metabolism, and phenylalanine metabolism having the lowest q values.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Duran et al. 1990[9]	Rat	Not defined	BUN (no change post-IR)	Kidney weight (increase post-IR)	Only measured AAs: The plasma concentrations of AAs were indistinguishable from control. Cellular AAs post-IR: glutamate, glycine, phenylalanine, and serine in cortical cell water (=derived from tissue) were decreased 3 h after ischemia, but those of the remaining 12 AAs were not different from control. Concentrations of glutamate and glycine had normalized 24 h after blood reflow, leaving only 4 AAs – arginine, phenylalanine, serine and threonine – at decreased concentration.
Gaudio et al. 1991[10]	Rat	EM images of proximal tubule segments: "Following ischemia and 15 min of reperfusion, there were marked cellular alterations typical of ischemic injury".	-	Histology	45 min of ischemia resulted in a significant fall in ATP levels. ATP levels had increased by only a small amount after 2h of reperfusion.
Huang et al. 2018[11]	Rat	Not defined	SCr (increase post-IR) Lactate (tissue and plasma levels increase post-IR)	Histology	Metabolomics: -Palmitate, stearate, linoleate, 1-monopalmitin, 2-monopalmitin, 2-monostearin, and cholesterol appeared to accumulate after 4 h followed by a reduction after 24 h IR. -Reduced glucose levels in both 4 h-IR and 4 h-control (4 h-C) kidneys that were sustained in 24 h-IR as compared to HC (healthy control). Glucose levels were unaltered in plasma of 4 h and 24 h-operated animals. Lactate levels were increased in 24 h-IR as compared to HC, and were also significantly elevated in the plasma of 4 h-operated animals and even to a greater extent after 24 h. -Blood creatinine levels were higher in both 4 h and 24 h post-IR. -Compensatory changes in metabolite levels in the uninjured organ of animals subjected to kidney IR, in particular after 24 h reperfusion: a strong elevation of urea and AMP in contralateral kidneys after 24 h post-IR, which was not observed in the injured kidney counterpart. Adenosine, glutamic acid and glycine levels were increased in a more prominent fashion in contralateral kidneys, particularly after 24 h. Citrate appeared to be elevated in all conditions as compared to control. ATP levels were significantly decreased in 24 h-IR kidney tissue as compared to 24 h-C and HC.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
					Integrated analysis: Hierarchical cluster analysis revealed the existence of five phenotypes: i) Decreased substrates in 4 h-IR and/or 4 h-C compared to HC and 24 h-IR/C. This includes proteins involved in fatty acid (FA) biosynthesis (Acsl6, Acsl4), metabolites involved in energy metabolism (glucose and citric acid); ii) Decreased substrates prevalently in 24 h-IR compared to the other conditions. This group: adenosine, proteins involved oxidation and reduction reactions (Por) and enzyme that play role in the TCA cycle (Pdha1/1). iii) Metabolites increased prevalently in 24 h-C animals as compared to the other conditions. This includes free fatty acids (FFAs) (2-Monostearin, 2-Monopalmitin and linoleate), non-essential amino acids (Glutamic acid, glycine), urea, AMP and creatinine. iv) Proteins and metabolites prevalently increased in 24 h-IR including enzymes involved in oxidative phosphorylation (Ndufa6, Ndufv1, and Ndufs1), fatty acid binding protein (Fabp4), glycolysis enzyme (Hk1) and lactate. v) Enzymes and metabolites elevated in 4 h-IR and 4 h-C such as glucose transporter (Slc5a1), FA transporter (CD36), components of oxidative phosphorylation (ND-1), detoxification enzymes (Adh5, Ugt2b15), mitochondrial biogenesis (Sirt2), FFA metabolism (Cpt1a, Acadsb, Echdc3, palmitate, stearate, 1-monopalmitin) and ketone metabolism (Oxct1).
Lan et al. 2016[12]	Rat	IR injury was monitored by SCr and renal histology. *Note: no thresholds given.*	SCr (no data reported)	Histology	During reperfusion 3, 7, and 14 days after IR, lactate and pyruvate concentrations in cortex and the outer stripe of outer medulla were increased relative to time controls.
Legouis et al. 2020[3]	Rat	Not defined	-	-	Renal gluconeogenesis, selectively quantified by analysis of blood from the renal vein, was reduced in response to IR.
Lindhardt et al. 2020[13]	Rat	Not defined	SCr (no control) BUN (no control) Urinary creatinine (no control)	-	The metabolism measured in vivo by product/pyruvate ratios showed a significant decrease in the ischemic kidney compared with the contralateral kidney when considering all metabolites (lactate, alanine, bicarbonate).

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Liu et al. 2012[14]	Rat	BUN and SCr were two widely used indicators of kidney Injury. There were significant differences in SCr and BUN between the control and IR groups. *Note: no thresholds given.*	SCr (increase post-IR) BUN (increase post-IR)	-	-Most important IR-related metabolites are lysophospholipids and FFAs, including stearoyl-glycerophosphocholine, eicosatrienoyl-glycerophosphocholine, oleoyl-glycerophosphocholine, palmitoyl-glycerophosphocholine, linoleoyl-glycerophosphocholine, linolenoyl lysolecithin, stearic acid, oleic acid, linoleic acid, arachidonic acid and eicosapentaenoic acid. -Nitrotyrosine (oxidative product of tyrosine) significantly increased in the IR group. -Increased hydroxymethylphenidate after IR. -Carnitine and acetyl-carnitine decreased during IR. -Saturated fatty acids and unsaturated fatty acids displayed different changes in the IR group. However, stearic acid made more contribution than polyunsaturated fatty acids (PUFAs) to discriminate between the IR and control.
Nielsen et al. 2017a[15]	Rat	Functional kidney parameters showed consistent signs of renal IR injury with an elevated plasma creatinine level of 91% and a reduced creatinine clearance and BUN level of 44% and 30%, respectively, when comparing pre-surgery with post-surgery values. *Note: no thresholds given.*	SCr (increase post-IR) BUN (increase post-IR) Creatine clearance (decrease post-IR) Urine output (non-significant increase post-IR)	Histology Body weight (non-significant decrease post-IR) Kidney weight (increase post-IR)	An elevated malate/fumarate ratio of 339% in the ischemic kidneys compared to that in the contralateral kidney was found.
Nielsen et al. 2017b[16]	Rat	We demonstrated that increased BUN and plasma creatinine occurred in both unilateral IR groups compared with sham-operated rats. Together, this indicates that renal IR resulted in acute renal insufficiency. *Note: no thresholds given.*	SCr (increase post-IR) BUN (increase post-IR)	Renal KIM-1 and NGAL mRNA expression (increase post-IR) Body weight (decrease post-IR) Kidney weight (increase post-IR (60 min ischemia))	Significant decrease of 18–25% in the pyruvate-to-lactate conversion in the 60-min postischemic kidney compared with the contralateral kidneys and kidneys from sham-operated rats. No reduction in pyruvate-lactate turnover was observed in the 30 min IR Group. The alanine-to-pyruvate and bicarbonate-to-pyruvate ratio similarly showed a decrease of 44% and 59%, respectively, in the postischemic kidney, no reduction in metabolite turnover was seen in the 30 min IR Group. The lactate-to-bicarbonate ratio was significantly shifted toward anaerobic glycolysis in the 60-min postischemic kidney by 44%. No statistical difference was found between alanine metabolism and aerobic glycolysis (alanine-to-bicarbonate ratio), but the lactate-to-alanine ratio was significantly increased by 25%, and a small increase in lactate-to-alanine ratio of 23% was also seen in the 30-min IR group. Pyruvate-to-total carbon signal and a total carbon kidney fraction were calculated for each kidney of the sham-operated and unilateral IR rats, which yielded a significantly elevated ratio of 6% for the 60-min postischemic kidney. Increase of lactate in the 60-min IR group of 178%, and no significant increase in the 30-min group.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Nielsen et al. 2020[17]	Rat	Not defined	SCr (increase post-IR for longer ischemia, stable levels post-IR for short ischemia, (partially) recover 7 days post-IR)	-	Acute alterations in the ischemic re-perfused kidneys overall metabolic phenotype were seen between the ischemic/early perfusion stage (2 min) and after 1 hour of perfusion, showing a compensatory mechanism in the contralateral kidney 60 min after reperfusion. The acute change in the lactate-to-bicarbonate ratio 60 min after reperfusion was not correlated with the early signature 2 min after reperfusion. The acute metabolic reprogramming seen at 60 min was driven by post release compensation in the contralateral kidney as well as downregulation of the lactate-to-bicarbonate balance in the ischemic kidney. The in vivo response that was seen 24 hours and 7 days following ischemic injury showed a similar tendency towards a general reduction of the overall metabolism in the ischemic kidney as well as a compensatory increased anaerobic metabolism shown by increased lactate production when compared to the aerobic metabolism, shown by CO2 and HCO3-production. Both 20 min and 40 min ischemia in the kidney results in a tendency towards a metabolic reprogramming from 24 hours to 7 days, with a statistically significant shift observed in the 40 min group. The metabolic phenotype at 24 hours, with reduced lactate-to-bicarbonate ratio, is positively correlated with the lactate-to-bicarbonate ratio at 7 days. A positive correlation was found in the lactate-to-bicarbonate ratio between 24 hours and 7 days. While no such correlation was found between the perfusion stage (2 min) and 60 min. By looking at the 20 min and 40 min group, one group with a large variance covering the 30 min acute insult, we compared the overall metabolic pattern from the initial 2 min – 7 days between the ischemic and contralateral kidney. This combination shows a significant change already at 60 min which persists throughout the 7 days. These findings are supported by a tendency towards a correlation between the lactate-to-bicarbonate ratio.
Peto et al. 2018[18]	Rat	Not defined	Lactate (blood lactate increase post-IR, pH decrease)	-	-Blood lactate concentration in parallel to pH increased by the end of the observed reperfusion period in all groups. In Control group the change was not significant, but in IR, where the largest rise was found, it was. -No significant change in glucose concentration.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Serkova et al. 2005[19]	Rat	Graft function: clinical appearance, histology, SCr, and urine output. *Note: no thresholds given.*	SCr (increase post-IR) Anuria	Histology Clinical appearance (behavior, fur, etc.) Body weight (no change post-IR)	<u>Tissue:</u> -Cold storage in UW solution: significant increase in glycogen and other carbohydrates was observed at 24 and 42 hours of CI. The lactate concentration was greatly increased, up to 380% during CI. In the lipid extracts, a decrease of PUFAs was seen in CI groups versus native kidney. There were no significant differences in metabolic composition at the end of 24 and 42 hours of CIT. -Transplanted kidneys exposed to reperfusion for 24 hours demonstrated characteristic changes: most pronounced difference between the post-IR and post-I groups was the dramatic increase of allantoin. Allantoin concentrations were low in the native kidney and at the end of CI, but increased significantly after 24 h reperfusion. Stepwise logistic regression analysis revealed that from 30 metabolites quantified from kidney extracts, only two–allantoin and PUFA – were different among study groups. <u>Blood:</u> -In 6/8 animals in the native group, allantoin concentrations were below the limit of quantification for NMR. - Allantoin peaks appeared in the blood in both CI groups following reperfusion. Allantoin concentrations were higher in transplanted rats with 42-hour cold storage when compared with 24-hour cold storage. -Trimethylamine-oxide (TMAO) correlated well with CIT. TMAO concentration correlated well with elevated allantoin levels. -Uric acid concentrations were below the linear range for the assay.
Shen et al. 2017[20]	Rat	Renal injury confirmed by histology: disrupted kidney structure.	-	Histology	-All metabolites enriched in the biosynthesis of unsaturated fatty acids were augmented in IR group in contrast to the control group. -D-glucose, lactic acid and cholesterol were differentiated significantly between control and IR group.
Tani et al. 2019[21]	Rat	Not defined	Lactate (tissue levels increase post-IR) SCr (increase post-IR) BUN (increase post-IR)	-	-Energy charge decreased upon ischemia, and increased upon reperfusion. -Total adenine nucleotide (TAN), ATP and ADP levels are decreased upon ischemia, and increased only slightly upon reperfusion. AMP showed hardly any difference between stationary state and ischemia, but decreased during reperfusion to a lower level. -Hypoxanthine, xanthine, and uric acid levels are high after ischemia, but return to baseline after reperfusion. -All groups in the ischemic state showed an increase in IMP level and a decrease in deoxyadenosine triphosphate (dATP) level, and a decrease in the adenine level after reperfusion. -TAN' (the sum of all detectable purine metabolites excluding ATP, ADP, and AMP, thus including dATP, PRPP, adenosine, adenine, inosine, IMP, hypoxanthine, xanthine, and uric acid) increased during ischemia and dropped to approximately baseline after reperfusion again. -Drastic metabolic changes by the IR procedure: triphosphate compounds in purine/pyrimidine metabolism pathways, nicotinamide adenine dinucleotide (NAD+), uridine diphosphate (UDP)-glucose, kynurenine, citrulline and amino acids such as ornithine, isoleucine, leucine, and tryptophan. -Marked accumulation of hydrolysis products, such as lactate and ß-hydroxybutyrate.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Trifillis et al. 1984[22]	Rat	SCr and BUN were used as indices of renal function. *Note: no thresholds given.*	SCr (increase post-IR) BUN (increase post-IR)	Survival	1 h ischemia and variable reperfusion times: ATP levels after 1 h of clamping decreased significantly to 18% of control levels. Upon release of the clamp, ATP levels began to increase after 0.25 h of reflow. ATP concentrations finally returned to control levels after 24 h of reflow. ADP levels remained relatively unchanged. AMP levels doubled after 1 h of ischemia but promptly returned to control levels after 0.25 h of reflow. Therefore, changes in AXP levels paralleled those of ATP levels, i.e., AXP levels were not fully restored to control levels until 24 h of reflow. The energy charge decreased to 50% of the control value after 1 h of ischemia but returned to control levels after 6 h of reflow. Lactate levels reached 13-fold control levels after 1 h of clamping and remained significantly elevated until 24 h of reflow when they returned to control levels. Variable ischemia times and 24 h of reperfusion: 120 min of ischemia resulted in death within the 24-h reflow period. SCr levels increased significantly after 60 and 90 min of ischemia followed by 24 h of reflow. In the left kidney, adenine nucleotides, lactate, and inorganic orthophosphate levels were restored essentially to control levels after 30 min of ischemia followed by 24 h of reflow. Adenine nucleotide levels were partially restored but remained significantly lower than control levels after 60 and 90 min of ischemia and 24 h of reflow. Lactate levels were restored to controls after 15-90 min of ischemia followed by 24 h of reflow. Inorganic orthophosphate and the phosphorylation state were significantly different from controls only after 60 min of ischemia followed by 24 h of reflow. Adenine nucleotide, lactate, and inorganic orthophosphate content of the right kidney were not significantly different from that of the left kidney at any time period studied, i.e., the time course and magnitude of metabolite restoration following 24 h of reflow was the same in both kidneys.
Varga et al. 2019[23]	Rat	Not defined	Lactate (serum levels increase post-IR, pH decrease) SCr (increase post-IR)	-	Lactate and potassium concentration significantly increased in IR (measurements after 120 min). IR decreased the pH.
Mouse					
Beier et al. 2020[24]	Mouse	Not defined	-	-	We found 30 metabolites elevated in IR. Among the uniquely increased metabolites in IR (compared to acute cellular rejection), the highest fold difference was observed for the lysine catabolite saccharopine. Also the downstream products 2-aminoadipate and glutarate, but not the parent substrates lysine and α-ketoglutarate, were increased in IR.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Chihanga et al. 2018[25]	Mouse	SCr, urinary NGAL, urinary NGAL/ urinary creatinine ratios, and urinary creatinine levels confirmed AKI 24 h post-IR. *Note: no thresholds given.*	SCr (increase post-IR) Urinary creatinine (decrease post-IR)	Histology Urinary NGAL (increase post-IR) Urinary NGAL/urinary creatinine (increase post-IR)	-Urinary concentrations of many metabolites before IR changed dramatically after IR. Cis-aconitate, citrate, creatine, phosphocreatine, putrescine, sarcosine, succinate, taurine, n-nitrosodimethylamine, trimethylamine, uracil, and trimethylamine N-oxide, galactaric acid, guanine and hippurate all decreased following IR. Nicotinamide-n-oxide, trigonelline, 2-oxoglutarate, and 2-oxoisocaproate were absent in urine following injury. Glucose, lactate, alanine, valine, and leucine had higher urine concentrations following IR. -NMR spectroscopy of plasma collected 24 h post-IR indicated no new metabolites post-IR except for creatinine. Metabolic profiling of kidney tissue extracts indicated no new metabolites following IR.
Cho et al. 2017[26]	Mouse	There was a significant increase in acute tubular injury in the IR group compared to the sham group.	-	Histology	-The levels of adenosine and 5'-deoxy-5'-methylthioadenosine were higher in IR-injured kidney. IR-injured kidney was characterized by decreased phosphatidylethanolamine (PE) 20:3/20:4, and betaine aldehyde levels in kidney samples, as well as increased cell membrane constituents. -Increased serum levels of fatty acids; in particular, 4,14-dimethyl-hexadecanoic acid, 15-eicosenoic acid, 3,5-dimethyl-tetradecanoic acid, cis-8,11,14-eicosatrienoic acid, and 3-oxo-2-pentyl-cyclopentaneoctanoic acid. The acyl-carnitines 2-octenoylcarnitine and 2-hydroxylauroyl-carnitine were decreased in the urine and serum, respectively. Levels of arachidonic acid and cis-4,7,10,13,16,19-docosahexaenoic were lower following IR than in the sham group.
*Chouchani et al. 2014[27]	Mouse	Not defined	-	-	Increase of hypoxanthine and xanthine after ischemia. Succinate accumulation during ischemia, recovery to baseline after 5 min of reperfusion.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Fujii et al. 2019[28]	Mouse	Transient ischemia for 10 min was sufficient to cause significant renal injury with increased NAG in urine and decreased creatinine clearance. No histological damage. *Note: no thresholds given.*	Creatinine clearance (decrease post-IR)	Histology (note: no changes) Urinary NAG (increase post-IR)	-In the normal kidney sections, high-energy adenine nucleotides (ATP and ADP) were significantly rich (vs inner medulla) in both the cortex and the outer medulla (outer stripe of outer medulla (OSOM) and inner stripe of outer medulla (ISOM)). -ATP and total adenylates in the cortex and OSOM decreased by transient ischemia for 10 minutes: -ATP in the cortex and the outer medulla decreased within a minute after the clipping procedure, and adenosine, inosine, and hypoxanthine increased in every region of the kidney. In particular, the content of ATP decreased by 45% within a minute of ischemia, and the decrease reached 84% during 10 min ischemia. In the inner medulla, ATP did not decrease within a minute, and significant decline first became evident at 10 min after clipping. AMP increased in every region of the kidney during the ischemia. Energy charge value decreased in the whole kidney within a minute. Accumulation of adenosine in the OSOM disappeared after the clipping procedure, which was associated with the increase of adenosine in regions of the kidney except for the OSOM. -Inosine and hypoxanthine increased in every region of the kidney within a minute and in parallel to AMP, and the degrading changes of adenylates progressed during 10 min ischemia. Metabolome analysis revealed increase of xanthine and uric acid in the ischemic kidney. -In the reperfusion sections, restoration of ATP in the renal cortex and OSOM was not complete, and ATP showed a 24% decrease when compared with sham sections. In contrast, the restoration of ATP in the ISOM and inner medulla was sufficient. Total adenylates in the cortex and OSOM decreased after 24 h reperfusion, total adenylates were maintained in the ISOM and inner medulla: total adenylates and ATP in the cortex and OSOM after 24 h reperfusion demonstrated prolonged loss. The breakdown products of ATP were increased in the whole kidney by 10 min ischemia, almost recovered to the original content after 24 h reperfusion. A prolonged loss of ATP was significant after 10 min ischemia. -NADH showed a 5-fold increase in all regions of the kidney subjected to 10 min ischemia. The increase in NADH in the cortex and OSOM persisted during 24 h of reperfusion, whereas it significantly decreased (vs 10 min ischemia) in the ISOM and inner medulla.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Jouret et al. 2016[29]	Mouse	The renal function was monitored by SCr and serum urea levels. IR-exposed mice showed a significant increase of both AKI parameters. *Note: no thresholds given.*	SCr (increase post-IR, recover 48 h post-IR) Serum urea (increase post-IR, recover 48 h post-IR) Lactate (urinary and tissue levels increase post-IR)	-	Urine: -Urine levels of taurine, lactate and glucose were steadily increased after IR, urine levels of trimethylamine were significantly reduced. -Pathways significantly affected by renal IR: gluconeogenesis and taurine/hypotaurine metabolism at 6 and 24h reperfusion. Protein biosynthesis, glycolysis and galactose and arginine metabolisms appeared essential at 48h reperfusion. Allantoin increased 24 h post-IR, but decreased 48 h after. Tissue: -Similar discriminations in tissue: changes in levels of lactate, fatty acids, choline and taurine. -The identification of metabolites, whose increased abundance reached significance in loading plots included fatty acids (and modified lipoproteins), lactate and N-acetyl groups of glycoproteins. -Levels of taurine and myo-inositol were decreased in kidneys from IR mice in comparison to sham animals. -Analysis of metabolites at 6h and 24h reperfusion: taurine/hypotaurine and betaine metabolisms were significantly affected by renal IR. -At 48h post reperfusion, IR-associated cascades were protein biosynthesis, biotin and taurine/hypotaurine metabolism. Serum: Serum analysis could not discriminate sham-operated from IR-exposed animals.
Legouis et al. 2020[3]	Mouse	Not defined	SCr (increase post-IR) BUN (increase post-IR)	-	Mice exposed to severe IR injury displayed increased urea and creatinine levels, together with a decrease in glucose and increase in lactate serum levels at 48 h, whereas urinary glucose and lactate were unchanged except for one outlier. Blood lactate clearance was impaired in the IR group after intraperitoneal injection of sodium lactate. Increase in blood glucose following lactate injection was reduced in the IR group.
Poyan Mehr et al. 2018[30]	Mouse	SCr was measured as a measure for renal function and postischemic injury. *Note: no thresholds given.*	SCr (increase post-IR)	-	204 metabolites measured, 27 were more than twofold increased in postischemic urines compared to controls including several sugars and amino acids, a pattern consistent with tubular impairment. Among these metabolites was quinolinate, an intermediate in the de novo NAD+ biosynthetic pathway from tryptophan. Many other metabolites, including amino acids and acyl-carnitines, were differentially regulated in urine of AKI mice.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Rao et al. 2016[31]	Mouse	Renal injury confirmed by SCr levels and histology. *Note: no thresholds given.*	SCr (increase post-IR)	Histology	Only measured lipids: -Four lipids were changed (all increases) to a statistically significant extent at 6 h after IR. Of these, three were identified as ether-linked phospholipids (one an abundant phosphatidylcholine (PC): PC O-38:1, and two PEs: an abundant PE O-42:3 and a minor PE O-40:4). The two abundant ether lipids were: PC O-38:1 (PC O-18:0, 20:1) and PE O-42:3 (PE O-20:1, 22:2). PC O-38:1 is a plasmanylcholine, while PE O-42:3 is a plasmalogen. -Many more lipids were changed to a statistically significant extent at 24 h after IR. The abundant PC O-38:1 remained elevated in IR kidneys at 24 h compared with the 24-h sham group, the low-abundance PE O-40:4 was present at comparably low levels in kidneys of both IR and sham mice at 24 h. PE O-42:3, was present at high levels but was decreased at 24 h compared with its sham control group, this lipid was increased relative to its sham control group at 6 h post-IR. All ether-linked PEs and PEs detected 24 h post-IR were reduced with AKI. -No statistically significant differences in major hydroxyoctadeca dienoic acids and hydroxyeicosatetra enoic acids or linoleic and arachidonic acids were detected in kidneys of sham and IR animals at 6 h post-IR.
Wei et al. 2014[32]	Mouse	Renal function: statistically significant differences in SCr or BUN levels compared to sham condition. *Note: no thresholds given.*	SCr (increase post-IR, peak 48 h post-IR, recover 1 week post-IR) BUN (increase post-IR, peak 48 h post-IR, recover 1 week post-IR) Lactate (tissue and serum levels decline post-IR, but recover)	-	-The changes started in renal cortex, followed by medulla and plasma. -Increased allantoin levels (specifically cortex, not medulla). -Elevated serum β-hydroxybutyrate levels. -The kidney cortex and the plasma samples showed early decreases in glucose and lactate, but recovery to near-sham levels by 1 week reperfusion time. -Some metabolites, such as 3-indoxyl sulfate, were induced at the earliest time point of renal IR. -There was a notable switch of energy source from glucose to lipids. -Decreased polyols for osmotic regulation. -Several pathways involved in inflammation regulation were induced. -Late induction of prostaglandins.
Zager et al. 2014[33]	Mouse	Severity of AKI was assessed by BUN and plasma creatinine concentrations and renal cortical NGAL mRNA levels. *Note: no thresholds given*	SCr (increase post-IR) BUN (increase post-IR)	-	Ischemia induced persistent pyruvate depletion. During ischemia, decreasing pyruvate levels correlated with increasing lactate levels. During early reperfusion, pyruvate levels remained depressed, but lactate levels fell below control levels. During late reperfusion, pyruvate depletion corresponded with increased gluconeogenesis (pyruvate consumption).

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Pig					
Clendenen et al. 2019[34]	Pig	Not defined	Lactate (serum levels increase post-IR) SCr (increase post-IR)	-	-Lactate increased in response to IR. Glutamate accumulation with a serum increase of 2.7 times from baseline occurring exclusively after reperfusion. Hypoxanthine increased 4 times baseline. IR changed arginine, proline, creatine and polyamine metabolism. Arginine decreased. Proline increased 1.1 times baseline following IR. Creatinine with increased 1.6 times baseline. Arginine consumption and accumulation of ornithine, polyamines (putrescine, spermidine and spermine) were observed upon IR. Spermine increased 5 times baseline levels, with spermidine following a similar trend. Putrescine increased. -Small molecule metabolites involved in redox homeostasis (e.g. reduced glutathione–GSH, cysteine, carnosine, kynurenine, taurine and hypotaurine) and, in general, metabolites involved in glutathione turnover (5-oxoproline) or sulphur metabolism (taurine, hypotaurine, methionine, GSH) were affected. Taurine increased upon reperfusion. No substantial increases in the post-IR circulating levels of carnitine, tryptophan and serotonin.
Fonouni et al. 2011[35]	Pig	Not defined	SCr (no “substantial” differences) BUN (no “substantial” differences) Lactate (extracellular fluid levels peak post-IR, but recover to baseline)	-	Baseline (BL) value = measured parameters in donors at the beginning of the graft procurement. <u>Baseline:</u> Glucose 0.56 mM, lactate 0.46 mM, pyruvate 12.17 μM, glutamate 19.75 mM, glycerol 19.58 μM <u>Procurement:</u> Glucose increase to 1.11 mM, lactate increase 0.54 mM, pyruvate increase 28.03 μM then decrease, glutamate increase to ~40 mM, glycerol similar to BL <u>CIT:</u> Glucose decrease to 0.23 mM, lactate decrease until halfway then increase to 0.35 mM, pyruvate short increase to 20.02 μM then decrease to 4.85 μM, glutamate increase to 82.60 mM, glycerol increase at end to 54.76 μM <u>WIT:</u> Glucose decrease 40 min to 0.14 mM then sharp increase to 0.48 mM, lactate increase to 0.75 mM, pyruvate increase to 10.18 μM, glutamate increase to 131 mM, glycerol increase to 118.22 μM <u>Reperfusion:</u> Glucose increase 40 min 1.47 mM then decrease to 0.73 mM, lactate increase 20 min to 1.07 mM then decrease to 0.58 mM, pyruvate increase 40 min to 29.97 μM then decrease to 17.80 μM, glutamate increase 20 min to 161.60 mM then decrease 40 min 41.03 mM then steady, glycerol increase 40 min to 236.70 μM then decrease to 19.60 μM

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Hauet et al. 2000[36]	Pig	Death: acute renal failure confirmed by histological analysis. These results were associated with prolonged oliguria or anuria. Assessment of renal function: creatinine clearance and fractional excretion of Na+ demonstrated reduced renal function. *Note: no thresholds given.*	Creatinine clearance (decrease post-IR, recover slightly)	Survival Urinary NAG excretion (increase post-IR, recover slightly) Fractional Na^+ excretion (increase post-IR, recover to near-baseline)	-The urinary lactate/Cr ratio was significantly greater in the IR groups vs control. -Urinary citrate/Cr level was higher in control vs ischemia.
Malagrino et al. 2019[37]	Pig	The animals showed changes characteristic of AKI: increased SCr, serum NGAL, fractional excretion of sodium, potassium and chloride and increased glucose and protein in urine. The most important result for the diagnosis to AKI was based on histological analysis, which showed acute tubular necrosis. An increase of nitrated protein in serum and urine was also observed. *Note: no thresholds given.*	SCr (increase post-IR)	Histology Serum NGAL (increase post-IR) Fractional Na^+, K^+ and Cl^- excretion (increase post-IR) Urinary glucose (increase post-IR) Urinary protein (increase post-IR)	<u>Serum:</u> -Metabolites that showed a quick increase or decrease after 60 min of ischemia, followed by a progressive return to baseline after reperfusion: L-glutamate, L-serine, N-isovaleroylglycine, L-methionine, L-proline, 2-aminobutyrate, and choline. These metabolites discriminate between the pre-ischemic and ischemia periods, as well as between the ischemic and 11 h post-reperfusion periods. However, as they returned to basal levels, they do not discriminate between the pre-ischemia and reperfusion periods <u>Urine:</u> Focus on urinary metabolites increased immediately after the reperfusion (0.5 h post-reperfusion), the result of "washing" the ischemic kidney. A sharp increase or decrease in 0.5 h post-reperfusion period compared with the pre-ischemia period: 3-hydroxybutyrate, 3-hydroxyisovalerate, methylguanidine, 3-aminoisobutyrate, trigonelline, betaine, glycerol, trimethylamine, carnosine, citrate, N-phenylacethylglycine, pyruvate and 1-methylnicotinamide. These metabolites were able to discriminate between the 0.5 h post-reperfusion and pre-ischemia periods. <u>Pathway and network analysis:</u> The metabolites identified were overrepresented in the canonical pathways of amino acids degradation, lipid metabolism, molecular transport, small molecule biochemistry, cell cycle, cellular assembly and organization.

Article	Species	Injury definition	Renal function clinical markers	Additional damage markers	Results on metabolome
Dog					
Maessen et al. 1989[38]	Dog	Post-transplant viability	-	Survival	Energy metabolites measured: ATP, ADP, AMP, GTP, GDP, GMP, IMP. CI with/without WI: -24 h CI: adenine nucleotide and guanine nucleotide contents decreased 30%. IMP increased. -48 h CI: no differences with 24 h CI. -30 min WI prior to CI: adenine and guanine nucleotide content already decreased before start of CI, increase of IMP. CI of 24 h after 30 min of WI did not affect content of adenine and guanine nucleotides, but IMP levels further increased. Prolonged CI to 48 h further decreased adenine and guanine nucleotide levels, while IMP did not show an additional increase. Reperfusion: 1h of reperfusion after 24 or 48 h of CI resulted in increase in guanine nucleotide pool. The adenine nucleotides remained unaffected in the 24 h group, but dropped in the 48 h group. IMP levels were greatly reduced in both. When 30 min WI was applied prior to CI, some different patterns were observed. In WI-groups, adenine nucleotide levels dropped in 24 h groups but remained unaffected in 48 h groups after 1 h reperfusion. The effect on guanine nucleotide and IMP levels did not differ from non-WI groups. Following reperfusion, non-WI groups showed higher (ATP+ADP)/AMP ratios.
Montanés et al. 1991[39]	Dog	Not defined	Creatinine clearance (decrease post-IR) BUN (increase post-IR) Lactate (systemic serum levels similar post-IR, kidney-specific clearance decreased post-IR)	-	Systemic blood: no significant changes in pH, bicarbonate, glutamine, glutamate, alanine, lactate, and pyruvate. Kidney specific (AV sampling): no significant changes in slutatmine, glutamate, alanine, and pyruvate. Decreased lactate clearance. Renal cortex: decreased glutamine, glutamate, ADP, AMP, and TAN. No significant changes in α-ketoglutarate, aspartate, lactate, pyruvate, alanine, and ATP. Urine: increased fractional excretion of lactate and pyruvate.

*Results are (partially) quoted or paraphrased from the texts. AA: amino acid. AC: acylcarnitine. AV: arterio-venous. BUN: blood urea nitrogen. CA: cardiac arrest. CPB: cardiopulmonary bypass. CI: cold ischemia. CIT: cold ischemia time. dATP: deoxyadenosine triphosphate. DGF: delayed graft function. FA: fatty acid. fDGF: functional DGF. FFA: free fatty acid. IR: ischemia reperfusion. ISOM: inner stripe of outer medulla. KIM-1: kidney injury molecule-1. NAG: N-acetyl-β-D-glucosaminidase. NGAL: neutrophil gelatinase-associated lipocalin. OSOM: outer stripe of outer medulla. PC: phosphatidylcholine. PE: phosphatidylethanolamine. Post-I: post-ischemia. Pre-I: pre-ischemia. PUFA: poly unsaturated fatty acids. SCr: serum creatinine. TMAO: trimethylamine-oxide. WI: warm ischemia. WIT: warm ischemia time. *: additionally included study.*

Table 4. An overview of the most commonly used definitions of delayed graft function (DGF) and acute kidney injury (AKI) in clinical practice, based on Mallon et al.[40] and Shin et al.[42] (GFR: glomerular filtration rate. RRT: renal replacement therapy.)

Delayed graft function[40]	
Most commonly used	Requirement for dialysis in the first postoperative week
Dialysis-based	Requirement for dialysis in the first postoperative week excluding the first 24 h
	Requirement for two or more episodes of dialysis in the first postoperative week
	Requirement for dialysis in the first 10 days postoperatively
Functional (creatinine-based)	Failure of a fall in serum creatinine of 10% on 3 consecutive days in the first postoperative week
	Serum creatinine at postoperative day 7 >2.5 mg/dL (=221 μM)
	Serum creatinine at postoperative day 10 >2.5 mg/dL (=221 μM)
	Fall in ratio of serum creatinine of postoperative days 1 and 2 of at least 30%
Combination	Dialysis in first week or failure of serum creatinine to fall in first 24 h
	Dialysis in the first week or serum creatinine at postoperative day 7 >2.5 mg/dL (=221 μM)

Acute kidney injury[42]						
RIFLE classification		*AKIN classification*		*KDIGO classification*		*All*
	Serum creatinine		*Serum creatinine*		*Serum creatinine*	*Urine output*
Risk	≥1.5 times baseline, or ≥25% decrease in GFR	Stage 1	≥0.3 mg/dl (=56.52 μM) increase, or ≥1.5 times baseline within 48 h	Stage 1	≥1.5-1.9 times baseline within 7 days, or ≥0.3 mg/dL (=56.52 μM) increase within 48 h	<0.5 mL/kg/h for >6 h
Injury	≥2 times baseline, or ≥50% decrease in GFR	Stage 2	≥2 times baseline	Stage 2	≥2.0-2.9 times baseline within 7 days	<0.5 mL/kg/h for 12 h
Failure	≥3 times baseline, or increase to ≥4 mg/dL, or ≥75% decrease in GFR	Stage 3	≥3 times baseline, or increase to ≥4.0 mg/dL (=353.6 μM) with acute increase of >0.5 mg/dL (=44.2 μM), or initiation of RRT	Stage 3	≥3 times baseline within 7 days, or increase to ≥4.0 mg/dL (=353.6 μM) with acute increase of >0.5 mg/dL (=44.2 μM), or initiation of RRT	<0.3 mL/kg/h for 24 h, or anuria for >12 h

due to their limited follow-up time (Table 2/3). In fact, most preclinical studies (24/35) relied on follow-up times of 24 hours or less, and in 11 studies the follow-up time was even limited to 120 minutes or less. Only 10/35 studies reported a follow-up of more than 24 hours, nine of which addressed aspects of recovery, including one study that exclusively relied on histology as an end-point. For one study, post-reperfusion follow-up time was not reported.

I/R injury was induced by unilateral clamping with the functional, contralateral kidney left in place in 14/35 studies, while 11/35 applied bilateral clamping, 5/35 performed unilateral nephrectomy prior to IR, and one induced transient cardiac arrest. A minority of studies (4/35) applied kidney transplantation as a model of I/R injury. Autotransplantation and contralateral nephrectomy following reimplantation were performed in two studies. One study transplanted an allograft in a bilaterally nephrectomized recipient, and one study did not report whether nephrectomy was performed.

Biomaterial sampling

Diverse sampling protocols (both with regard to the type of biomaterial collected and the timing of the sampling) are applied among the studies (Table 2). The majority of studies (24/27) that include blood sampling, relied on peripheral blood samples (9/24 studies did not specify sampling location). Organ-specific measurements using renal vein blood was performed in 3/27 studies: in rats, pigs and dogs. One of the peripheral blood sampling studies performed kidney-specific sampling through microdialysis. Single post-reperfusion sampling was reported in 13/27 studies. All other studies concerned multiple sampling (two or more consecutive post-reperfusion blood samples).

Eleven studies included urine samples. Urinary sampling was generally (6/11) achieved by means of metabolic cages, and consequently cumulative samples were reported. One porcine and dog study sampled directly from the bladder or ureter, respectively. Three studies did not specify the means of urine collection.

Most studies (17/28) that included tissue-based analysis for metabolomic profiling or as a read-out of injury were based on a single sampling point, both for smaller and larger animals. Studies reporting serial timepoints all relied on biopsies from separate animals. The majority of the studies (18/28) used whole organs, 10/28 (including two studies that also applied *in vivo* magnetic resonance imaging (MRI)) performed a region-specific analysis (i.e. cortex and medulla).

A large variety of *ex vivo* and *in vivo* analysis platforms was used among the studies. *Ex vivo* analysis for metabolic strategies was diverse and included mass spectrometry and/or nuclear magnetic resonance (NMR)-based platforms, as well as more traditional biochemical assays (gas/liquid chromatography and/or enzymatic techniques). *In vivo* measurements were performed in 4/35 studies (all rat), and concerned ^{1}H-MRI combined with hyperpolarized ^{13}C-MRI.

An extensive metabolomic profile was included in 16/35 studies. Other studies applied a focused (targeted) approach, and reported (sub-)aspects of the metabolome, e.g. exclusively amino acids, lipids or high energy phosphates. For the sake of clarity, extracted data was clustered along the lines of metabolic competence (energy (high energy phosphate) and/or redox status), and the primary metabolic routes (glycolysis,

tricarboxylic acid (TCA) cycle, fatty acid oxidation (β-oxidation), amino acids) to allow for comparison of metabolic signatures.

Metabolic outcomes

Metabolic aspects (i.e. reported contrasts between cases and controls) from each experimental study are summarized in a qualitative overview (Figure 2; quantitative data was categorized to minimize interference caused by differences in measurement techniques and normal values). Data regarding metabolic recovery was only available for studies reporting successive timepoints (Table 2).

Reported metabolic profiles for experimental I/R injury are diffuse, and often incomplete (Figure 2). For example, the metabolic clues required to assess post-reperfusion metabolic (in)competence (i.e. information on energy equivalents) were only available for a subset (10/35) of studies. Based on these studies, most (6/7) rodent studies and the dog studies indicate post-reperfusion metabolic competence (i.e. recovery of high energy phosphates and/or absent release of products indicating ATP/GTP degeneration). Elevated levels of high energy phosphate breakdown products were reported in one pig study (5-minutes post-reperfusion), but no information was available for later timepoints; it is therefore unclear whether this reflects post-reperfusion wash-out or I/R injury.[4] Reported aspects of the post-reperfusion redox status (tissue acetoacetate/β-hydroxybutyrate, or lactate/pyruvate ratio (plasma, serum, dialysate)) and their dynamics following experimental IR vary substantially.

Tissue and blood glucose levels are generally reported to be decreased or recovering after experimental IR (Figure 2). Blood lactate levels are mostly increased following IR, while its tissue contents are reported variably. Among rodent studies, remarkable variations were observed in the dynamics of TCA cycle intermediates.[8,20] The limited data for porcine studies is relatively consistent, generally showing increased circulating levels of TCA intermediates following IR.

Aspects of β-oxidation were only reported in a minority (n=10) of rodent studies, and conclusions were variable (Figure 2). Similarly, reported aspects of amino acid metabolism and intermediates are limited and conclusions are variable: tissue levels are decreased or stable following IR in rats and dogs, whilst levels in murine tissues are highly variable.

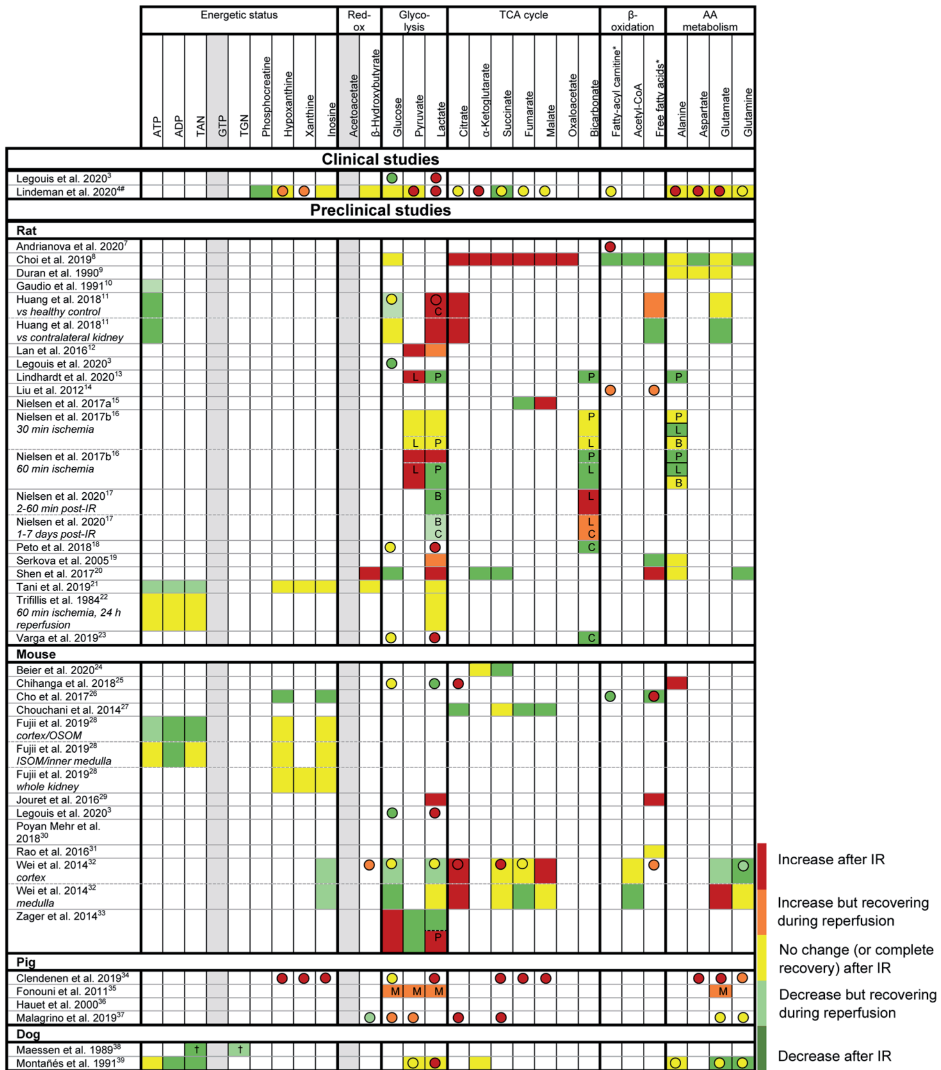


Figure 2. Relative differences in renal tissue (boxes) and blood (spheres, note: often systemic if no data on kidney-specific samples) metabolite levels between control kidneys (healthy/intact control, sham-surgery control, or contralateral kidney: see Table 2) and kidneys after ischemia reperfusion (IR). If no control data was available, data was excluded. Metabolites marked in grey were not reported for tissue or blood samples in any of the preclinical studies. Red = increase after IR. Orange = increase but recovering during reperfusion (requires multiple samples during reperfusion). Yellow = no change (or complete recovery) after IR. Light green = decrease but recovering during reperfusion (requires multiple samples during reperfusion). Green = decrease after IR. TAN: total adenine nucleotides (ATP+ADP+AMP). TGN: total guanine nucleotides (GTP+GDP+GMP). *: variety of fatty acids, results on overall trend. #: additional data on tissue levels is reported in the supplementary data, but no formal statistical evaluation was performed. OSOM: outer stripes of outer medulla. ISOM: inner stripes of outer medulla. †: ischemia time-dependent. P: metabolite/pyruvate ratio. L: metabolite/lactate ratio. B: metabolite/bicarbonate ratio. C: in control (sham-operated animal or contralateral kidney) metabolite levels showed similar dynamics following surgery. M: measured using microdialysis.

Discussion

Recent clinical leads for DGF and AKI point towards a universal discriminatory metabolic profile for, and possibly causal role of, metabolic aspects in renal I/R injury.[3,4] This observation is remarkable considering the obvious mechanistic differences between DGF (warm and cold ischemia) and AKI (exclusively warm ischemia), and may imply a universal mechanism for clinical renal I/R injury. It was reasoned that these observations provide a lead for validation of preclinical models of I/R injury. A systematic review was performed to align the observed metabolic profiles for clinical context with those reported in preclinical studies. This review shows poor alignment of the reported preclinical metabolic data with clinical evidence, and identifies several critical methodological shortcomings in the preclinical studies.

Whilst the phenomenon of I/R injury has been known for over 50 years, a persistent translational gap in transforming the abundant preclinical therapeutic successes towards a clinical benefit remains. In fact, 10 years ago, partners in the NIH CAESAR consortium (in the context of myocardial I/R injury) stated that: "for 40 years, the National Heart, Lung, and Blood Institute has invested enormous resources (at least several hundred million dollars) in preclinical studies aimed at developing infarct-sparing therapies, and several hundred (if not thousands) therapies have been claimed to limit infarct size in preclinical models. Unfortunately, due largely to methodological problems, this enormous investment has not produced any notable clinical application".[1] Similar conclusions were expressed with respect to renal I/R injury.[2,43]

Two recent clinical studies positioned metabolic defects in the center of renal I/R injury.[3,4] Although the conclusions of the studies do not allow discrimination between a causative mechanism or secondary defect, findings are fully discriminatory and provide insight in the early events of clinical I/R injury, and imply a metabolic mechanism as driver of clinical I/R injury. Alignment of the preclinical models with the clinical context identified a number of critical issues that may fundamentally interfere with translation of preclinical findings.

A first, fundamental aspect concerns the diagnosis/definition 'I/R injury'. Clinical studies not only identified a clear signature for I/R injury, but also indicated graded degrees of recovery in the absence of I/R injury, i.e. in the context of transplantation an almost instantaneous functional and metabolic recovery for living donor grafts versus a more suspended recovery for deceased donor grafts (Figure 1).[4] Although less detailed information was available for AKI, different grades of AKI also associate with graded metabolic recoveries (e.g. KDIGO stage 3, demonstrating more severely impaired metabolism compared to less severe, i.e. KDIGO stage 1, injury).[3]

Clinical I/R injury (DGF) was accompanied by a fully discriminatory reperfusion metabolome, an aspect that allowed for discrimination between I/R injury and IR damage. To be specific: I/R injury is the injury triggered by the metabolic paralysis, with persistent ATP catabolism despite adequate reperfusion. IR *damage* on the other hand, reflects tissue damage caused by entry of cellular and/or humoral effectors upon reestablishment of blood flow, processes that are independent from the metabolic cataplexy and may occur in response to an ischemic insult as well as I/R injury.

A further aspect identified in the review were the notable variations in techniques applied to induce IR, ischemia times, timing of the post-reperfusion sampling, and considerable heterogeneity concerning species, strains and gender used. Considering the established interspecies, -strain, and sex variations in metabolism, the metabolic susceptibility, [44-47] and the impact of environmental factors on metabolic flexibility,[7,48,49] this heterogeneity may contribute to the compromised interchangeability of research findings.

Most (23/35) preclinical reports relied on serum creatinine as functional read-out of kidney function/injury. Group-wise comparisons (intervention versus control), and single or short (≤24 hours) follow-up times were generally applied. Consequently, appropriate ischemic controls and/or recovery profiles series required for discrimination between IR damage and injury were absent in preclinical studies. Moreover, conclusions with respect to creatinine clearance from preclinical studies that relied on cardiac arrest, bilateral clamping, or on unilateral clamping/transplantation with unilateral nephrectomy are potentially interfered by a secondary prerenal kidney insufficiency resulting from a uremia-induced somnolence with suppressed thirst reflex. Potential secondary prerenal kidney insufficiency is avoided in protocols applying unilateral clamping without contralateral nephrectomy (14/35 studies) (which obviously interferes with the use of creatinine clearance as read-out) or possibly by renal replacement therapy (none of the identified studies). No study applied prespecified serum creatinine thresholds to grade the degree of injury, or corrected for over- or dehydration during or following surgery.
A broad palette of histological, plasma/serum, and/or urinary markers was used to further grade kidney injury. Since multiple evaluations concluded that histological examinations poorly predict outcome,[50,51] the question arises whether a strong reliance on histology is justified; in fact, one preclinical study reported I/R injury based on serum creatinine levels in absence of histological damage.[28]
Reliance on, or the inclusion of, urinary samples in some studies is remarkable, considering that transient anuria is a key characteristic of clinical renal I/R injury. Consequently, it is unlikely that the injury sustained in these studies reflects the degree of injury in clinical I/R injury.

The majority of preclinical studies (23/27) that include plasma/serum measurements exclusively relied on peripheral samples for metabolic profiling (Table 2). Interpretation of this data is potentially interfered by the physiologic clearance mechanisms.[4] In fact, metabolic signals are fully absent in the peripheral (arterial) blood samples in the clinical setting, and selective post-renal venous sampling was required for distinctive signals. [4]
Tissue metabolites are reported by 25/35 studies. Although metabolic information from tissue samples can be highly informative, this approach is only appropriate for non-diffusible metabolites; a parallel evaluation of metabolic data from tissue biopsies and renal venous samples indicated wash-out of diffusible metabolic intermediates, with stable tissue contents. [4]

Alignment of reported metabolic profiles identified contrasting findings between preclinical and clinical studies. Whilst clinical I/R injury (DGF) is characterized by energetic impairment that persists beyond the 30-minutes post-reperfusion measurement-window (Table 1)[4], reported data from rodent/dog studies implies reinstatement of energy equivalents following reperfusion. Hence, observations in these models align with the observations for grafts without I/R injury.
Selective arteriovenous sampling identified normoxic glycolysis and impaired lactate handling as key characteristics of clinical renal I/R injury (Table 1).[3,4] Only three experimental studies applied selective venous sampling,[3,34,39] but very limited metabolic analysis was performed.
A specific TCA cycle defect at the level of the oxoglutarate-dehydrogenase complex with selective post-reperfusion release of α-ketoglutarate and impaired recovery of tissue succinate levels was identified in clinical I/R injury (DGF) (Table 1). This finding contrasts with the metabolome of rodent I/R injury, which is reportedly associated with an ischemic accumulation of succinate and its subsequent rapid re-oxidation during reperfusion, paralleled by an oxidative burst.[27] The latter rapidly subsides upon normalization of succinate levels (within 5 minutes of reperfusion).[27] This brief insult contrasts with the prolonged, but reversible metabolic defects in clinical I/R injury (Table 1, Figure 1). Although it cannot be excluded that a succinate-driven mechanism contributes to the initiation of I/R injury, the transient character and the apparent rapid metabolic recovery contrast with the clinical context.

In conclusion, this systematic evaluation based on reported metabolic aspects of I/R injury demonstrates profound methodological variability and shortcomings in the preclinical studies. Whilst some of these limitations are inherent to preclinical research, e.g. overall interspecies differences and differences in resilience, most shortcomings can be circumvented. A key issue in preclinical studies is the inability to discriminate between IR damage and I/R injury. Additional challenges in preclinical research are the strong impact of age and comorbidities on incidental AKI and DGF[52-54] (an aspect that is largely

ignored in preclinical studies that generally use young and healthy animals), the need for kidney-specific sampling, prolonged follow-up and bridging renal support to quantify the actual impact of IR. Finally, reported metabolic aspects are diffuse and often scattered. Consequently, studies to date do not allow for adequate metabolic phenotyping and/or comparison of outcomes. To fully capture the complex metabolic interactions, animal models are essential for research. Recommendations to improve the translatability of preclinical research are therefore summarized in Table 5 and Figure 3.

This systemic review has some limitations. The available data is limited. Only two clinical studies are available, one concerning DGF and one on AKI. Although there is consensus that they both reflect renal I/R injury, these are obviously distinct entities. Preclinical reports were extremely heterogenous, hence no formal meta-analysis could be performed. This study is kidney-focused; given the organ-specific difference in metabolism and metabolic rates, observations may not directly translate to other tissues.

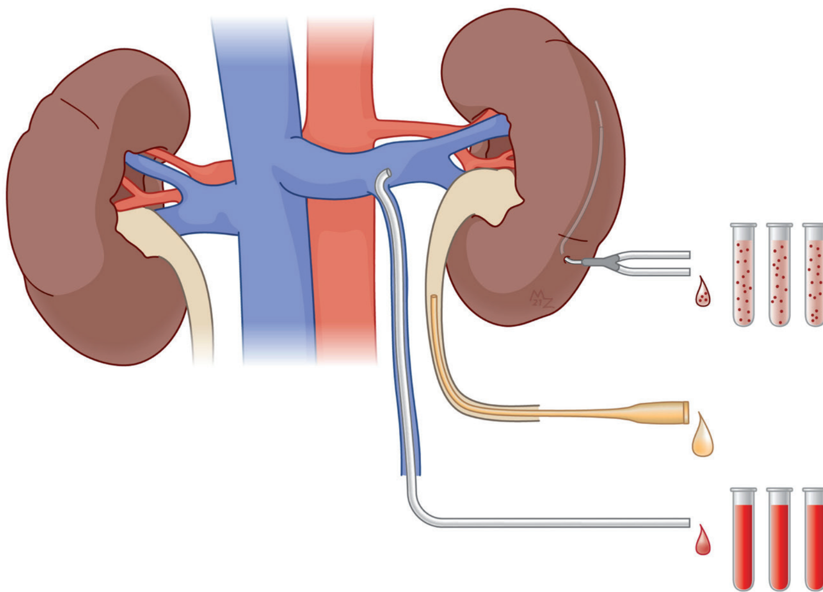


Figure 3. Recommendations to improve the translatability of preclinical models of renal ischemia reperfusion (IR) injury. Ischemic injury should be induced unilaterally in order to avoid interference caused by uremia. Appropriate discrimination between IR damage and IR injury critically relies on the organ-specific assessment of metabolic competence (i.e. prolonged normoxic glycolysis, see outline in Figure 1). Kidney-specific metabolic profiling of the injured kidney can be achieved through renal-vein specific blood sampling using the spermatic vein as access.[39,55] Microdialysis is a potential alternative to arteriovenous sampling, but has not yet been validated for this purpose. Ureterostomy[56] allows for selective functional monitoring of the injured kidney. The model critically relies on optimized ischemia times in order to achieve actual IR injury and avoid excess incidences of IR damage or non-function. It is anticipated that successful implementation of these prerequisites relies on use of rats or larger laboratory animals. Illustration by Manon Zuurmond.

Table 5. Recommendations for future preclinical studies investigating renal metabolism following ischemia reperfusion (IR).

Recommendation	Rationale
Functional IR injury definition	Consensus on renal dysfunction
Renal replacement therapy	Exclusion of prerenal causes of renal IR injury
Optimal ischemia time (titration of IR injury)	Chance for both transient dysfunction, i.e. DGF, and ischemic controls, i.e. primary function
Representative study population	Higher incidence of clinical renal IR injury in older patients
Renal vein catheterization (excludes use of mice)	Arteriovenous kidney-specific sampling; metabolite diffusion
Successive sampling	Monitoring dysfunction and functional recovery
Extended follow-up time	Monitoring dysfunction and functional recovery
Predefined set of metabolites	Insight in metabolic competence, straightforward comparisons

Supplemental data

1.1 Literature search

Two separate systematic literature searches were performed in the databases of PubMed, EMBASE and Web of Science, in which the literature until December 2020 was examined.

Studies were excluded if 1) no preclinical (i.e. animal) model was applied; 2) only organs other than kidneys were examined; 3) only ischemia was applied, in the absence of reperfusion; 4) data was solely gathered after ischemia rather than after IR; 5) only metabolite levels of compounds that are not directly involved in energy metabolism were measured (e.g. phospholipids, drug metabolites); 6) metabolites were not measured (e.g. only enzyme activities); 7) kidney injury was due to other causes than IR (e.g. rejection, toxins); 8) it concerned a review, book section or case report; 9) language was other than English.

Altogether, 35 preclinical studies were included from the systematic searches (Supplementary Figure 1a and 1b) and were subsequently clustered according to their study population (i.e. species studied). Details of the searches in PubMed, EMBASE and Web of Science are given under *1.2 Search components*.

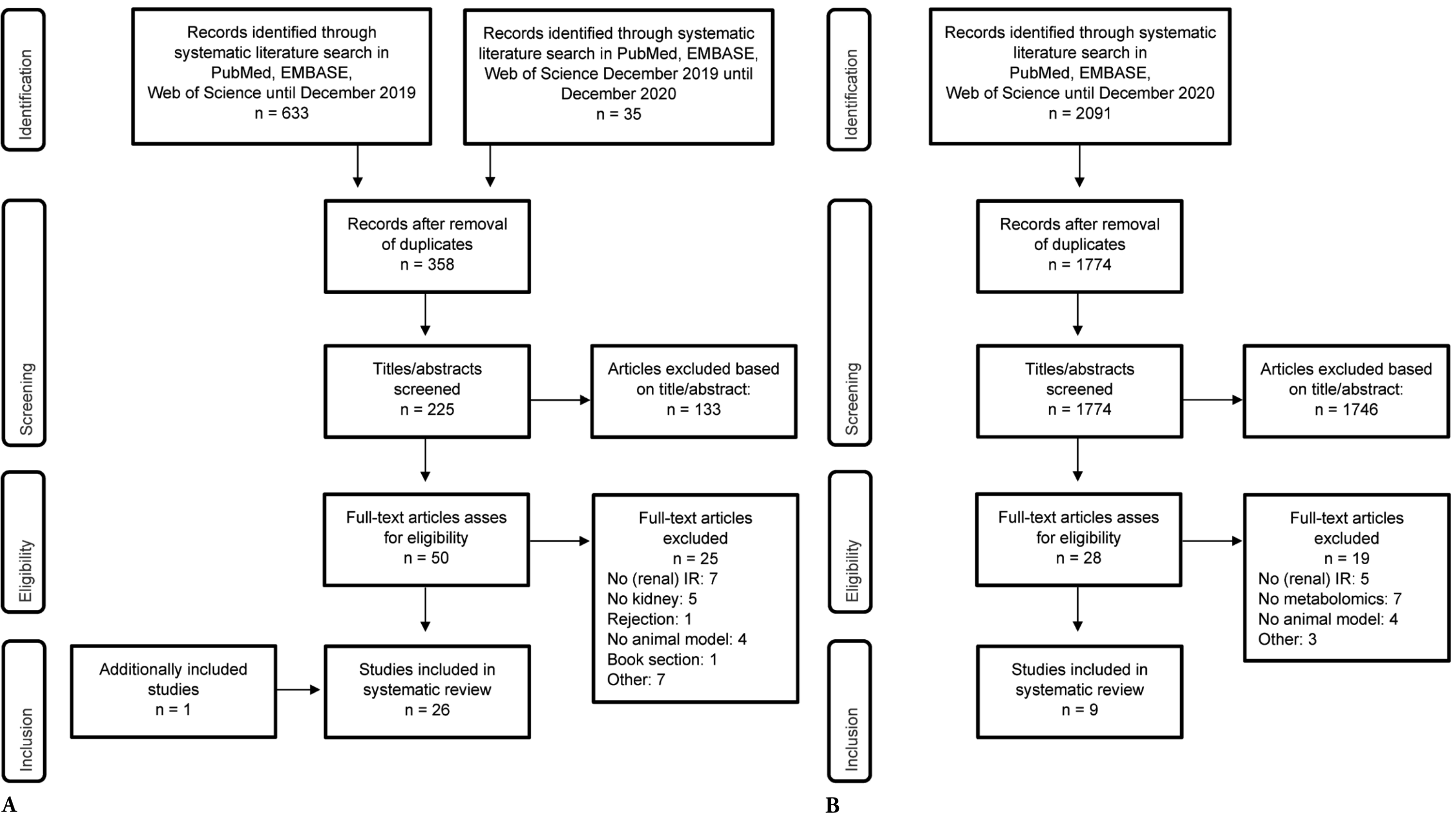
1.2 Search components

1.2.1 DGF search

In total, 220, 15 and 88 unique references were found using systematic searches in respectively Pubmed, EMBASE and Web of Science on the 19th of December, 2019, excluding duplicates. Additionally, 22, 5 and 8 unique references were found using systematic searches in respectively Pubmed, EMBASE and Web of Science on the 1st of December, 2020, excluding duplicates as well as references identified by the DGF search from December 2019.

PubMed

((“ischemia-reperfusion”[tw] OR ischemia-reperfus[tw] OR “ischaemia-reperfusion”[tw] OR ischaemia-reperfus*[tw] OR “Reperfusion Injury”[Mesh:noexp] OR “reperfusion injury”[tw] OR “reperfusion injuries”[tw] OR reperfusion injur*[tw] OR “reperfusion damage”[tw] OR reperfusion damag*[tw] OR “ischemic injury”[tw] OR “ischemic injuries”[tw] OR ischemic injur*[tw] OR “ischaemic injury”[tw] OR “ischaemic injuries”[tw] OR ischaemic injur*[tw] OR “Primary Graft Dysfunction”[mesh] OR “primary graft dysfunction”[tw] OR “primary graft failure”[tw] OR “Delayed Graft Function”[Mesh] OR “delayed graft function”[tw]) AND (“Kidney”[mesh] OR “kidney”[tw] OR “kidneys”[tw] OR “renal”[tw] OR “Kidney transplantation”[mesh] OR kidney transplant*[tw] OR “renal transplantation”[tw] OR renal transplant*[tw] OR kidney graft*[tw] OR renal graft*[tw] OR “renal injury”[tw] OR “renal injuries”[tw] OR “kidney injury”[tw] OR “kidney injuries”[tw]) AND (“Metabolomics”[Mesh]*



Supplemental Figure 1. Flowchart of the systematic literature search performed to identify preclinical studies reporting metabolic data following renal ischemia reperfusion (IR) in the context of a) Delayed Graft Function (DGF) and b) Acute Kidney Injury (AKI).

OR "Metabolome"[mesh] OR "metabonomics"[tw] OR "metabolomics"[tw] OR metabonom[tw] OR metabolom*[tw] OR "metabolic profile"[tw] OR "metabolic profiles"[tw] OR "metabolites"[tw] OR "metabolite"[tw] OR "metabolic"[ti]) AND ("english"[la] OR "dutch"[la] OR "russian"[la]))*

EMBASE

(("ischemia-reperfusion".ti,ab OR "ischemia-reperfus".ti,ab OR "ischaemia-reperfusion".ti,ab OR ischaemia-reperfus*.ti,ab OR *"Reperfusion Injury"/ OR "reperfusion injury".ti,ab OR "reperfusion injuries".ti,ab OR "reperfusion injur*".ti,ab OR "reperfusion damage".ti,ab OR reperfusion damag*.ti,ab OR "ischemic injury".ti,ab OR "ischemic injuries".ti,ab OR ischemic injur*.ti,ab OR "ischaemic injury".ti,ab OR "ischaemic injuries".ti,ab OR ischaemic injur*.ti,ab OR *"Primary Graft Dysfunction"/ OR "primary graft dysfunction".ti,ab OR "primary graft failure".ti,ab OR *"Delayed Graft Function"/ OR "delayed graft function".ti,ab) AND (exp *"Kidney"/ OR "kidney".ti,ab OR "kidneys".ti,ab OR "renal".ti,ab OR exp "Kidney transplantation"/ OR kidney transplant*.ti,ab OR "renal transplantation".ti,ab OR renal transplant*.ti,ab OR kidney graft*.ti,ab OR renal graft*.ti,ab OR "renal injury".ti,ab OR "renal injuries".ti,ab OR "kidney injury".ti,ab OR "kidney injuries".ti,ab) AND (*"Metabolomics"/ OR *"Metabolome"/ OR "metabonomics".ti,ab OR "metabolomics".ti,ab OR metabonom*.ti,ab OR metabolom*.ti,ab OR "metabolic profile".ti,ab OR "metabolic profiles".ti,ab OR "metabolites".ti,ab OR "metabolite".ti,ab OR "metabolic".ti) AND ("english".la OR "dutch".la OR "russian".la))*

Web of Science

(ti=("ischemia-reperfusion" OR "ischemia-reperfus" OR "ischaemia-reperfusion" OR "ischaemia-reperfus*" OR "Reperfusion Injury" OR "reperfusion injury" OR "reperfusion injuries" OR "reperfusion injur*" OR "reperfusion damage" OR "reperfusion damag*" OR "ischemic injury" OR "ischemic injuries" OR "ischemic injur*" OR "ischaemic injury" OR "ischaemic injuries" OR "ischaemic injur*" OR "Primary Graft Dysfunction" OR "primary graft dysfunction" OR "primary graft failure" OR "Delayed Graft Function" OR "delayed graft function") AND ts=("Kidney" OR "kidney" OR "kidneys" OR "renal" OR exp "Kidney transplantation" OR "kidney transplant*" OR "renal transplantation" OR "renal transplant*" OR "kidney graft*" OR "renal graft*" OR "renal injury" OR "renal injuries" OR "kidney injury" OR "kidney injuries") AND (ts=("Metabolomics" OR "Metabolome" OR "metabonomics" OR "metabolomics" OR metabonom* OR metabolom* OR "metabolic profile" OR "metabolic profiles" OR "metabolites" OR "metabolite") OR ti="metabolic") AND la=("english" OR "dutch" OR "russian")) OR (ts=("ischemia-reperfusion" OR "ischemia-reperfus*" OR "ischaemia-reperfusion" OR "ischaemia-reperfus*" OR "Reperfusion Injury" OR "reperfusion injury" OR "reperfusion injuries" OR "reperfusion injur*" OR "reperfusion damage" OR "reperfusion damag*" OR "ischemic injury" OR "ischemic injuries" OR "ischemic injur*" OR "ischaemic injury" OR "ischaemic injuries" OR "ischaemic injur*" OR "Primary Graft*

Dysfunction" OR "primary graft dysfunction" OR "primary graft failure" OR "Delayed Graft Function" OR "delayed graft function") AND ti=("Kidney" OR "kidney" OR "kidneys" OR "renal" OR exp "Kidney transplantation" OR "kidney transplant" OR "renal transplantation" OR "renal transplant*" OR "kidney graft*" OR "renal graft*" OR "renal injury" OR "renal injuries" OR "kidney injury" OR "kidney injuries") AND (ts=("Metabolomics" OR "Metabolome" OR "metabonomics" OR "metabolomics" OR metabonom* OR metabolom* OR "metabolic profile" OR "metabolic profiles" OR "metabolites" OR "metabolite") OR ti="metabolic") AND la=("english" OR "dutch" OR "russian"))*

1.2.2 AKI search

In total, 1662, 92 and 20 unique references were found using systematic searches in respectively Pubmed, EMBASE and Web of Science on the 9th of December, 2020, excluding duplicates as well as references identified in the DGF search.

PubMed

(("Acute Kidney Injury"[majr] OR "Acute Kidney Injury"[ti] OR "Acute Kidney Injuries"[ti] OR "Acute Renal Injury"[ti] OR "Acute Renal Injuries"[ti] OR "Acute Kidney Failure"[ti] OR "Acute Kidney Insufficiency"[ti] OR "Acute Renal Failure"[ti] OR "Acute Renal Insufficiency"[ti] OR "AKI"[ti]) AND ("Metabolomics"[majr] OR "Metabolome"[majr] OR "metabonomics"[ti] OR "metabolomics"[ti] OR metabonom[ti] OR metabolom*[ti] OR "metabolic profile"[ti] OR "metabolic profiles"[ti] OR "metabolites"[ti] OR "metabolite"[ti] OR "Metabolism"[majr:noexp] OR "metabolism"[ti] OR "metabo*"[ti] OR ("Acute Kidney Injury/metabolism"[majr] AND "metabolism"[Subheading:NoExp])) AND ("english"[la] OR "dutch"[la] OR "russian"[la]) NOT (("Cisplatin"[Mesh] OR "cisplatin"[tw] OR "Sepsis"[Mesh] OR "sepsis"[tw] OR "toxic kidney"[tw] OR "toxic renal"[tw] OR "nephrotox*"[tw]) NOT ("surgery"[subheading] OR "Surgical Procedures, Operative"[Mesh] OR "surgery"[tw] OR "surgical"[tw] OR "surgical*"[tw] OR "Perioperative Period"[Mesh] OR "Perioperative Care"[Mesh] OR "peri operative"[tw] OR "perioperative"[tw] OR "Postoperative Period"[Mesh] OR "Postoperative Complications"[Mesh] OR "post operative"[tw] OR "postoperative"[tw] OR "Endovascular Procedures"[Mesh] OR "endovascular"[tw] OR "endo vascular"[tw])) NOT (("Review"[ptyp] OR "review"[ti]) NOT ("Clinical Study"[ptyp] OR "trial"[ti] OR "RCT"[ti] OR "Case Reports"[ptyp] OR "case report"[ti])))*

EMBASE

(("Acute Kidney Failure"/ OR "Acute Kidney Injury".ti OR "Acute Kidney Injuries".ti OR "Acute Renal Injury".ti OR "Acute Renal Injuries".ti OR "Acute Kidney Failure".ti OR "Acute Kidney Insufficiency".ti OR "Acute Renal Failure".ti OR "Acute Renal Insufficiency".ti OR "AKI".ti) AND (exp *"Metabolomics"/ OR exp *"Metabolome"/ OR "metabonomics".ti OR "metabolomics".ti OR metabonom*.ti OR metabolom*.ti OR "metabolic profile".ti OR "metabolic profiles".ti OR "metabolites".ti OR *"metabolite"/ OR "metabolite".ti OR*

**"Metabolism"/ OR "metabolism".ti OR "metabo*".ti) AND ("english".la OR "dutch".la OR "russian".la) NOT ((exp *"Cisplatin"/ OR "cisplatin".ti OR exp "Sepsis"/ OR "sepsis".ti OR "toxic kidney".ti OR "toxic renal".ti OR "nephrotox*".ti) NOT (exp "surgery"/ OR "su".fs OR "surgery".ti,ab OR "surgical".ti,ab OR "surgical*".ti,ab OR exp "Perioperative Period"/ OR "peri operative".ti,ab OR "perioperative".ti,ab OR exp "Postoperative Period"/ OR exp "Postoperative Complication"/ OR "post operative".ti,ab OR "postoperative".ti,ab OR "Endovascular Surgery"/ OR "endovascular".ti,ab OR "endo vascular".ti,ab)) NOT ((exp "Review"/ OR "review".ti) NOT (exp "Clinical Study"/ OR exp "Clinical trial"/ OR "trial".ti OR "RCT".ti OR "Case Report"/ OR "case report".ti)))*

Web of Science
(ti=("Acute Kidney Failure" OR "Acute Kidney Injury" OR "Acute Kidney Injuries" OR "Acute Renal Injury" OR "Acute Renal Injuries" OR "Acute Kidney Failure" OR "Acute Kidney Insufficiency" OR "Acute Renal Failure" OR "Acute Renal Insufficiency" OR "AKI") AND ti=("Metabolomics" OR "Metabolome" OR "metabonomics" OR "metabolomics" OR metabonom OR metabolom* OR "metabolic profile" OR "metabolic profiles" OR "metabolites" OR "metabolite" OR "metabolite" OR "Metabolism" OR "metabolism" OR "metabo*") AND la=("english" OR "dutch" OR "russian") NOT (ti=("Cisplatin" OR "cisplatin" OR "Sepsis" OR "sepsis" OR "toxic kidney" OR "toxic renal" OR "nephrotox*") NOT ts=("surgery" OR "surgery" OR "surgical" OR "surgical*" OR "Perioperative Period" OR "peri operative" OR "perioperative" OR "Postoperative Period" OR "Postoperative Complication" OR "post operative" OR "postoperative" OR "Endovascular Surgery" OR "endovascular" OR "endo vascular")) NOT ((TI="Review" OR DT="review") NOT TI=("Clinical Study" OR "Clinical trial" OR "trial" OR "RCT" OR "Case Report" OR "case series")))*

1.3 Data collection

One author collected data from the included studies. Extracted data included specifics concerning the study populations, control groups, intervention techniques, sampling techniques, measurement techniques, applied definitions of I/R injury, as well as metabolic data (i.e. reported metabolite levels). If controls were missing, metabolic data was excluded. To present the metabolic data of the studies, reported means of measured metabolite levels in study groups and control groups within single studies were collected and compared. All extracted data is publicly available in the cited publications.

1.4 Synthesis of results

Studies were considered eligible for inclusion of outcomes if they reported metabolic aspects, which is also a prerequisite according to the inclusion criteria. The data (i.e. reported metabolite levels) were mapped along theoretical pathways (energetic status, redox, glycolysis, TCA cycle, β-oxidation, amino acid metabolism) to obtain insight into changes in the major metabolic routes. Absolute measures were converted to semi-

quantitative data (i.e. dynamics of changes), which enabled direct comparisons between studies, despite great variety in applied methods. The studies were clustered according to their study population (i.e. type of animal studied). Altogether, this resulted in an illustrative overview in the form of a heatmap.

1.5 Risk of bias and sensitivity analysis of results

In this systematic review, we did not assess risk of bias of the included preclinical studies, as this is inherent to preclinical studies and their great heterogeneity (i.e. differences in species, strains, induction of injury). This excessive heterogeneity and concomitant granulated data also restricted the assessment of robustness of results, and results were therefore converted to qualitative aspects in a theoretical framework.

1.6 Statistics

This systematic review is a qualitative and descriptive study, and the reported quantitative data has already been statistically examined in the original publications. Therefore, further statistical tests were not applied.

1.7 Other information

The review was not registered. A review protocol was not prepared, as this study concerns an explorative meta-analysis. The PRISMA checklist for systematic reviews is given in Supplementary Table 1.

Acknowledgements

We would like to acknowledge LUMC librarian Jan W. Schoones for his dedicated support concerning the development of the literature search queries.

Supporting Table 1. PRISMA guidelines for systematic reviews checklist.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Supp. Info 1.1
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supp. Info 1.2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Supp. Info 1.3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Supp. Info 1.3
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Supp. Info 1.3
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	NA
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Supp. Info 1.3

Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Supp. Info 1.4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Supp. Info 1.4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Supp. Info 1.4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Supp. Info 1.4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Supp. Info 1.4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	10-16
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	10-16
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	6, Supp. Info 1.1-1.2
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Supp. Info Fig. 1
Study characteristics	17	Cite each included study and present its characteristics.	Table 2+3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supp. Info 1.5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Fig. 1

Section and Topic	Item #	Checklist item	Location where item is reported
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	10-16
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Supp. Info 1.6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6-9
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Supp. Info 1.5
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	10-16
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7-11
	23b	Discuss any limitations of the evidence included in the review.	16
	23c	Discuss any limitations of the review processes used.	16
	23d	Discuss implications of the results for practice, policy, and future research.	10-16
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Supp. Info 1.7
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	16
Competing interests	26	Declare any competing interests of review authors.	16
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supp. Info 1.3

References

1. Lefer DJ, Bolli R. Development of an NIH Consortium for PreclinicAl AssESsment of CARdioprotective Therapies (CAESAR): A paradigm shift in studies of infarct size limitation. J Cardiovasc Pharmacol Ther. 2011;16(3-4):332-339. doi:10.1177/1074248411414155
2. Cavaillé-Coll M, Bala S, Velidedeoglu E, et al. Summary of FDA workshop on ischemia reperfusion injury in kidney transplantation. In: American Journal of Transplantation. Vol 13. Am J Transplant; 2013:1134-1148. doi:10.1111/ajt.12210
3. Legouis D, Ricksten SE, Faivre A, et al. Altered proximal tubular cell glucose metabolism during acute kidney injury is associated with mortality. Nat Metab. 2020. doi:10.1038/s42255-020-0238-1
4. Lindeman JH, Wijermars LG, Kostidis S, et al. Results of an explorative clinical evaluation suggest immediate and persistent post-reperfusion metabolic paralysis drives kidney ischemia reperfusion injury. Kidney Int. August 2020. doi:10.1016/j.kint.2020.07.026
5. Van Os S, De Abreu R, Hopman J, Wethly K, Liem D, Van De Bor M. Purine and pyrimidine metabolism and electrocortical brain activity during hypoxemia in near-term lambs. Pediatr Res. 2004;55(6):1018-1025. doi:10.1203/01.PDR.0000125261.99069.D5
6. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6(7). doi:10.1371/journal.pmed.1000097
7. Andrianova N V., Popkov VA, Klimenko NS, et al. Microbiome-metabolome signature of acute kidney injury. Metabolites. 2020;10(4):142. doi:10.3390/metabo10040142
8. Choi J, Shoaib M, Yin T, et al. Tissue-Specific Metabolic Profiles After Prolonged Cardiac Arrest Reveal Brain Metabolome Dysfunction Predominantly After Resuscitation. J Am Heart Assoc. 2019;8(17):e012809. doi:10.1161/JAHA.119.012809
9. Duran MA, Spencer D, Weise M, Kronfol NO, Spencer RF, Oken DE. Renal epithelial amino acid concentrations in mercury-induced and postischemic acute renal failure. Toxicol Appl Pharmacol. 1990;105(2):183-194. doi:10.1016/0041-008x(90)90180-3
10. Gaudio KM, Thulin G, Ardito T, Kashgarian M, Siegel NJ. Redistribution of cellular energy following renal ischemia. Pediatr Nephrol. 1991;5(5):591-596. doi:10.1007/BF00856647
11. Huang H, van Dullemen LFA, Akhtar MZ, et al. Proteo-metabolomics reveals compensation between ischemic and non-injured contralateral kidneys after reperfusion. Sci Rep. 2018;8(1):8539. Published 2018 Jun 4. doi:10.1038/s41598-018-26804-8
12. Lan R, Geng H, Singha PK, et al. Mitochondrial Pathology and Glycolytic Shift during Proximal Tubule Atrophy after Ischemic AKI. J Am Soc Nephrol. 2016;27(11):3356-3367. doi:10.1681/ASN.2015020177
13. Lindhardt JL, Nielsen PM, Hansen ESS, et al. The hemodynamic and metabolic effects of spironolactone treatment in acute kidney injury assessed by hyperpolarized MRI. NMR Biomed. 2020;33(10):e4371. doi:10.1002/nbm.4371
14. Liu Y, Yan S, Ji C, et al. Metabolomic changes and protective effect of (L)-carnitine in rat kidney ischemia/reperfusion injury. Kidney Blood Press Res. 2012;35(5):373-381. doi:10.1159/000336171

15. Nielsen PM, Eldirdiri A, Bertelsen LB, Jørgensen HS, Ardenkjaer-Larsen JH, Laustsen C. Fumarase activity: an in vivo and in vitro biomarker for acute kidney injury. Sci Rep. 2017;7:40812. Published 2017 Jan 17. doi:10.1038/srep40812
16. Nielsen PM, Laustsen C, Bertelsen LB, et al. In situ lactate dehydrogenase activity: a novel renal cortical imaging biomarker of tubular injury?. Am J Physiol Renal Physiol. 2017;312(3):F465-F473. doi:10.1152/ajprenal.00561.2015
17. Nielsen PM, Qi H, Bertelsen LB, Laustsen C. Metabolic reprogramming associated with progression of renal ischemia reperfusion injury assessed with hyperpolarized [1-13C]pyruvate. Sci Rep. 2020;10(1):8915. Published 2020 Jun 2. doi:10.1038/s41598-020-65816-1
18. Peto K, Nemeth N, Mester A, et al. Hemorheological and metabolic consequences of renal ischemia-reperfusion and their modulation by N,N-dimethyl-tryptamine on a rat model. Clin Hemorheol Microcirc. 2018;70(1):107-117. doi:10.3233/CH-170361
19. Serkova N, Fuller TF, Klawitter J, Freise CE, Niemann CU. H-NMR-based metabolic signatures of mild and severe ischemia/reperfusion injury in rat kidney transplants. Kidney Int. 2005;67(3):1142-1151. doi:10.1111/j.1523-1755.2005.00181.x
20. Shen S, Wang JF, Wu JQ, et al. GC/MS-based metabolomic analysis of alleviated renal ischemia-reperfusion injury induced by remote ischemic preconditioning. Eur Rev Med Pharmacol Sci. 2017;21(4):765-774
21. Tani T, Okamoto K, Fujiwara M, Katayama A, Tsuruoka S. Metabolomics analysis elucidates unique influences on purine / pyrimidine metabolism by xanthine oxidoreductase inhibitors in a rat model of renal ischemia-reperfusion injury. Mol Med. 2019;25(1):40. Published 2019 Aug 22. doi:10.1186/s10020-019-0109-y
22. Trifillis AL, Kahng MW, Cowley RA, Trump BF. Metabolic studies of postischemic acute renal failure in the rat. Exp Mol Pathol. 1984;40(2):155-168. doi:10.1016/0014-4800(84)90073-x
23. Varga G, Ghanem S, Szabo B, et al. Renal ischemia-reperfusion-induced metabolic and micro-rheological alterations and their modulation by remote organ ischemic preconditioning protocols in the rat. Clin Hemorheol Microcirc. 2019;71(2):225-236. doi:10.3233/CH-189414
24. Beier UH, Hartung EA, Concors S, et al. Tissue metabolic profiling shows that saccharopine accumulates during renal ischemic-reperfusion injury, while kynurenine and itaconate accumulate in renal allograft rejection. Metabolomics. 2020;16(5):65. Published 2020 May 4. doi:10.1007/s11306-020-01682-2
25. Chihanga T, Ma Q, Nicholson JD, et al. NMR spectroscopy and electron microscopy identification of metabolic and ultrastructural changes to the kidney following ischemia-reperfusion injury. Am J Physiol Renal Physiol. 2018;314(2):F154-F166. doi:10.1152/ajprenal.00363.2017
26. Cho K, Min SI, Ahn S, et al. Integrative Analysis of Renal Ischemia/Reperfusion Injury and Remote Ischemic Preconditioning in Mice. J Proteome Res. 2017;16(8):2877-2886. doi:10.1021/acs.jproteome.7b00167
27. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature. 2014;515(7527):431-435. doi:10.1038/nature13909

28. Fujii K, Kubo A, Miyashita K, et al. Xanthine oxidase inhibitor ameliorates postischemic renal injury in mice by promoting resynthesis of adenine nucleotides. JCI Insight. 2019;4(22):1-20. doi:10.1172/jci.insight.124816
29. Jouret F, Leenders J, Poma L, Defraigne JO, Krzesinski JM, de Tullio P. Nuclear Magnetic Resonance Metabolomic Profiling of Mouse Kidney, Urine and Serum Following Renal Ischemia/Reperfusion Injury. PLoS One. 2016;11(9):e0163021. Published 2016 Sep 22. doi:10.1371/journal.pone.0163021
30. Poyan Mehr A, Tran MT, Ralto KM, et al. De novo NAD+ biosynthetic impairment in acute kidney injury in humans. Nat Med. 2018;24(9):1351-1359. doi:10.1038/s41591-018-0138-z
31. Rao S, Walters KB, Wilson L, et al. Early lipid changes in acute kidney injury using SWATH lipidomics coupled with MALDI tissue imaging. Am J Physiol Renal Physiol. 2016;310(10):F1136-F1147. doi:10.1152/ajprenal.00100.2016
32. Wei Q, Xiao X, Fogle P, Dong Z. Changes in metabolic profiles during acute kidney injury and recovery following ischemia/reperfusion. PLoS One. 2014;9(9):e106647. Published 2014 Sep 5. doi:10.1371/journal.pone.0106647
33. Zager RA, Johnson AC, Becker K. Renal cortical pyruvate depletion during AKI. J Am Soc Nephrol. 2014;25(5):998-1012. doi:10.1681/ASN.2013070791
34. Clendenen N, Nunns GR, Moore EE, et al. Selective organ ischaemia/reperfusion identifies liver as the key driver of the post-injury plasma metabolome derangements. Blood Transfus. 2019;17(5):347-356. doi:10.2450/2018.0188-18
35. Fonouni H, Esmaeilzadeh M, Jarahian P, et al. Early detection of metabolic changes using microdialysis during and after experimental kidney transplantation in a porcine model. Surg Innov. 2011;18(4):321-328. doi:10.1177/1553350610392063
36. Hauet T, Baumert H, Gibelin H, et al. Noninvasive monitoring of citrate, acetate, lactate, and renal medullary osmolyte excretion in urine as biomarkers of exposure to ischemic reperfusion injury. Cryobiology. 2000;41(4):280-291. doi:10.1006/cryo.2000.2291
37. Malagrino PA, Venturini G, Yogi PS, et al. Metabolomic characterization of renal ischemia and reperfusion in a swine model. Life Sci. 2016;156:57-67. doi:10.1016/j.lfs.2016.05.025
38. Maessen JG, van der Vusse GJ, Vork M, Kootstra G. The beneficial effect of intermediate normothermic perfusion during cold storage of ischemically injured kidneys. A study of renal nucleotide homeostasis during hypothermia in the dog. Transplantation. 1989;47(3):409-414. doi:10.1097/00007890-198903000-00001
39. Montañés I, Badía A, Réngel MA, López-Novoa JM. Renal cortical intermediary metabolism in the recovery phase of postischemic acute renal failure in the dog. Proc Soc Exp Biol Med. 1992;199(3):321-326. doi:10.3181/00379727-199-43363
40. Mallon DH, Summers DM, Bradley JA, Pettigrew GJ. Defining delayed graft function after renal transplantation: Simplest is best. Transplantation. 2013;96(10):885-889. doi:10.1097/TP.0b013e3182a19348
41. Ronco C, Bellomo R, Kellum JA. Acute Kidney Injury. Lancet. 2019;394(10212):1949-1964. doi:https://doi.org/10.1016/S0140-6736(19)32563-2

42. Shin SR, Kim WH, Kim DJ, Shin IW, Sohn JT. Prediction and Prevention of Acute Kidney Injury after Cardiac Surgery. Biomed Res Int. 2016;2016:2985148. doi:10.1155/2016/2985148
43. Saat TC, van den Akker EK, IJzermans JNM, Dor FJMF, de Bruin RWF. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: Lost in translation? J Transl Med. 2016;14(1). doi:10.1186/s12967-016-0767-2
44. Montgomery MK, Fiveash CE, Braude JP, et al. Disparate metabolic response to fructose feeding between different mouse strains. Sci Rep. 2015;5(1):18474. doi:10.1038/srep18474
45. Fleischer S, Sharkey M, Mealey K, Ostrander EA, Martinez M. Pharmacogenetic and metabolic differences between dog breeds: Their impact on canine medicine and the use of the dog as a preclinical animal model. AAPS J. 2008;10(1):110-119. doi:10.1208/s12248-008-9011-1
46. Strohl KP, Thomas AJ, St. Jean P, Schlenker EH, Koletsky RJ, Schork NJ. Ventilation and metabolism among rat strains. J Appl Physiol. 1997;82(1):317-323. doi:10.1152/jappl.1997.82.1.317
47. Reifsnyder PC, Te A, Harrison DE. Differential Effects of Rapamycin on Glucose Metabolism in Nine Inbred Strains. Lamming D, ed. Journals Gerontol Ser A. 2020;75(1):50-57. doi:10.1093/gerona/glz157
48. Habbout A, Li N, Rochette L, Vergely C. Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences. J Nutr. 2013;143(5):553-562. doi:10.3945/jn.112.172825
49. Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic Flexibility as an Adaptation to Energy Resources and Requirements in Health and Disease. Endocr Rev. 2018;39(4):489-517. doi:10.1210/er.2017-00211
50. Wang CJ, Wetmore JB, Crary GS, Kasiske BL. The donor kidney biopsy and its implications in predicting graft outcomes: A systematic review. Am J Transplant. 2015;15(7):1903-1914. doi:10.1111/ajt.13213
51. Hall IE, Reese PP, Weng FL, et al. Preimplant histologic acute tubular necrosis and allograft outcomes. Clin J Am Soc Nephrol. 2014;9(3):573-582. doi:10.2215/CJN.08270813
52. O'Sullivan ED, Hughes J, Ferenbach DA. Renal aging: Causes and consequences. J Am Soc Nephrol. 2017;28(2):407-420. doi:10.1681/ASN.2015121308
53. Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. Am J Transplant. 2010;10(10):2279-2286. doi:10.1111/j.1600-6143.2010.03179.
54. da Cruz PRC, Filho ACD, Santana VBBM, Boaretto RBB, Riccetto CLZ. Donor age amplifies the detrimental effects of cold ischemia time on long-term kidney allograft survival independently of the occurrence of delayed graft function or early acute rejection. Exp Clin Transplant. 2020;18(4):436-443. doi:10.6002/ect.2020.0066
55. Suzuki Y, Eto T. Androgens in testicular venous blood in the adult rat. Endocrinol Jpn. 1962;9:277-283. doi:10.1507/endocrj1954.9.277
56. Wang T, Yu Z, Chen C, et al. Ureteral anastomosis with a polyimide stent in rat kidney transplantation. Ren Fail. 2020;42(1):193-199. doi:10.1080/0886022X.2020.1726386