Unravelling metabolism during kidney perfusion using tracer studies, a systematic review

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Acknowledgments: We would like to thank Thomas Vandendriessche, Kristel Paque, and Krizia Tuand, the biomedical reference librarians of the KU Leuven Libraries – 2Bergen – Learning Centre Désiré Collen (Leuven, Belgium) for their help in conducting the systematic literature search. We would also like to thank Veerle Heedfeld for helping with protocol registration at Open Science Framework and Tine Wylin for help with the data extraction table. J.D.B. holds a PhD fellowship

This is the version of the paper as accepted by "Artifical Organs", doi: 10.1111/aor.14355
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fundamental research (1152820N) from The Research Foundation Flanders (FWO). This research received no external funding.

Author Contributions: Conceptualization, I.J.; methodology, I.J., J.D.B., B.G.; investigation, A.GV., R.V.D.; data curation, A.GV, R.V.D., J.D.B.; writing—original draft preparation, A.GV., R.V.D., J.D.B.; writing—review and editing, B.G., I.J.; visualization, A.GV., R.V.D., I.J.; supervision, I.J. All authors have read and agreed to the published version of the manuscript.

Running title: Tracer metabolomics in kidney perfusion

Word count: 5626

Abstract

Background:

Understanding kidney metabolism during perfusion is vital to further develop the technology as a preservation, viability assessment, and resuscitation platform. We reviewed the evidence on the use of labelled metabolites (tracers) to understand “on-pump” kidney behavior.

Methods:

PubMed, Embase, Web of Science, and Cochrane databases were systematically searched for studies evaluating metabolism of (non)radioactively labelled endogenous compounds during kidney perfusion.

Results:

Of 5899 articles, 30 were included. All were animal studies [rat (70%), dog (13%), pig (10%), rabbit (7%)] perfusing but not transplanting kidneys. Perfusion took place at hypothermic (4°C-12°C) (20%), normothermic (35°C-40°C) (77%), or undefined temperatures (3%). Hypothermic perfusion used albumin or a clinical kidney preservation solution, mostly in the presence of oxygen. Normothermic perfusion was mostly performed with oxygenated crystalloids often containing glucose and amino acids with unclear partial oxygen tensions. Active metabolism of carbohydrate, amino acid, lipids, and large molecules was shown in hypothermic and normothermic perfusion. Production of macromolecules, such as prostaglandin, thromboxane, and vitamin D, takes place during normothermic perfusion. No experiments compared differences in metabolic activity between hypothermic and normothermic perfusion. One conference abstract showed increased anaerobic metabolism in kidneys donated after circulatory death by adding labelled glucose to hypothermically perfused human kidneys.

Conclusions:

Tracer studies during kidney perfusion contribute to unravelling kidney metabolic behavior in pre-clinical models. Whether findings are truly translational needs further investigation in large animal

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models of human kidneys. Furthermore, it is essential to better understand how ischemia changes this metabolic behavior.

Keywords: isolated organ perfusion; kidney perfusion; machine perfusion; kidney; kidney transplantation; kidney metabolism; organ preservation; viability assessment; systematic review; isotopic tracer

Introduction

Improved organ preservation and options for viability assessment and resuscitation are direly needed to reduce post-transplant complications and avoid futile discard of deceased donor kidneys [1-3]. *Ex situ* kidney perfusion, also called machine perfusion, has been proposed as a platform that could accommodate all three unmet needs and is expected to be increasingly implemented in clinical settings [4-6].

While hypothermic perfusion has been shown to be superior to static cold storage, it is not a reliable viability assessment tool [7]. Good quality studies have shown that selection criteria investigated to date do not have adequate predictive power for decision-making [8-11]. The usefulness of newer markers, such as flavin mononucleotide, is the subject of ongoing research [12, 13]. Recent evidence shows hypothermic perfusion can be improved by actively adding oxygen and thereby likely promoting kidney metabolism during preservation, though the underlying mechanisms of action are not understood [14, 15]. Initial clinical experience with a short period of normothermic perfusion to 'resuscitate' the kidney following static cold storage, shows feasibility and a first large randomized controlled trial is awaited [16-19]. As the kidney is metabolically active at normothermic temperatures, it is believed that this platform is better suited for viability assessment and resuscitation compared to hypothermic perfusion [5, 11]. Nevertheless, no validated viability markers in kidney have been identified, unlike for liver where more evidence is available [4, 11].

As the kidney remains metabolically active during both hypothermic and normothermic perfusion, it need adequate metabolic support and it is surprising how little we know about the kidney's metabolic behavior during perfusion [20]. Furthermore, changes of the metabolome might also correlate with post-transplant outcomes and as such serve as viability markers. Adding a (non)radioactively labelled compound [21] – called a "tracer" – to the *ex situ* kidney perfusion set-up might provide insight into active or perturbed metabolic pathways and as such reveal information about the biochemical fate of different metabolites in the perfusate. Indeed, these labelled compounds are processed through the

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metabolic network and the label eventually ends up in products that are released into the perfusate. The way these products are labelled provides a non-invasive readout of the biochemical activities that gave rise to the observed labelling pattern. This allows to identify metabolic pathways that are actively connected to the parent labelled compound (Figure 1).

This systematic review assesses what tracer studies have revealed in the setting of hypothermic and normothermic kidney perfusion.

Methods

Search strategy

This review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol of this systematic review was prospectively registered in Open Science Framework [22]. With the help of an experienced biomedical information specialist, a search strategy was built and PubMed, Embase, Web of Science Core Collection, and Cochrane databases were searched. The following concepts: "tracer", "kidney", and "perfusion" were developed. The complete search strategy can be found in Table S1.

Study Selection, eligibility criteria, and study outcomes

Two authors independently assessed eligibility of the articles based on title and abstract, conducted full-text analysis, and extracted data. In case of disagreements, a third experienced researcher was consulted. Studies were included from database inception with final searches carried out on 30 May 2022. Studies were eligible for inclusion if they reported on any of the prespecified outcomes. Only studies in mammals evaluating the metabolism of the isolated kidney (both *ex situ* and *in situ*) were included. Articles were excluded when the metabolites were exogenous or not labelled. Articles written in a language other than English, Dutch, or French; articles with no full text available; review articles; letters; editorials; and conference abstracts were also excluded. Reference lists of included

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studies were also searched using the same inclusion and exclusion criteria ('snowballing') Since no study in human kidneys was identified, we ran an additional search in Embase on May 30 2022, with the specific aim to find published conference abstracts on tracer studies in perfused human kidneys (Table S1).

Data extraction

The results of the search were imported into Endnote (Version X9 or 20, Clearview Analytics, Philadelphia, PA, USA) where they were screened for duplicates by two independent reviewers. Duplicates were removed using the "Find duplicates" tool in Endnote. The remaining articles were imported to Rayyan [23] and screened according to prespecified inclusion and exclusion criteria (Table S2). The full data extraction table is publicly accessible and contains information on title, authors, year of publication, study type, experimental set-up, group characteristics, perfusion characteristics, tracer and labelling conditions, analyses, perfusate results, urine results, tissue results and isolated tubules results [24]. When details on experimental set-up were not mentioned, we attempted to retrieve information from referenced studies. Radioactive tracers are written between brackets (e.g. [^{14}C]).

Perfusion temperature was defined as hypothermic (0-12°C) or normothermic (>34°C) as in clinical practice and results are categorized accordingly [25].

Quality assessment

Quality was rated by two independent reviewers. The 'systematic review center for laboratory animal experimentation (SYRCLE's) risk of bias tool for animal studies' was used to assess the quality of the reported experiments and the article. This tool is based on the Cochrane Risk of Bias tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies [26]. Signaling questions were formulated by Hooijmans *et al.* to facilitate judgment and were reported to enhance transparency and applicability of the results [26].

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Results

Search Results

A systematic search of online databases, performed on May 30th, 2022, resulted in the identification of 9386 articles (PubMed: 3191, Embase: 3984, Web of Science: 1943, and Cochrane Library: 268). After duplicate removal, 5899 articles remained of which 5825 articles were excluded based upon predefined in and exclusion criteria at time of Title and Abstract screening (217 based on language, 106 based on type of publication, and 4834 based on content). Another 42 articles were excluded at time of full text screening, leaving 25 articles that were included. From the reference lists, another 771 potential papers were identified. After removal of 97 duplicates, an additional 669 records were excluded, leading to 5 additional inclusions (Figure 2). In total, 30 papers were included in this systematic review. The full data extraction table can be accessed online [24].

All articles reported on pre-clinical experimental studies and were published between 1974 and 2020. Most articles (25/30, 83%) were published between 1974 and 1992 followed by 14 years where no study could be identified. Five (5/30, 17%) articles were published more recently, between 2006 and 2020. Kidneys were retrieved from rats (21/30, 70%), rabbits (2/30, 7%), dogs (4/30, 13%), and more recently from pigs (3/30, 10%). A perfusion device, allowing *ex situ* kidney perfusion was used in all but four cases [27-51]. In the latter, the kidney was perfused *in situ* [52-54] or the perfusion method was unclear [55]. Kidney perfusion took place at hypothermic (4°C-12°C) (6/30, 20%) or normothermic temperatures (35°C-40°C) (23/30, 77%). In one study, studying glutamate metabolism by adding L-^{14}C-glutamine to the perfusion solution of rat kidneys, temperature was not further specified [56]. There were no studies in which the kidney was transplanted after perfusion.

An additional search was carried out on May 30th, 2022 to identify Conference Abstracts of tracer studies of perfused human kidneys registered in Embase. This search identified 174 records of which 1 was retained (Figure S1).

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Quality and risk of bias assessment

The majority of studies (28/30, 93%) compared at least two experimental groups. The risk of bias was assessed as "unclear" because essential information was often not reported (Figure 3, Table S3, S4). Risk of selection bias remained unclear for the majority of studies (21/30, 70%) [27-30, 32-34, 36, 37, 39, 41, 43-47, 49, 52-54] and could be rated as low in two studies [42, 50] and high in seven [31, 35, 38, 40, 48, 51, 55]. Group allocation was not reported in 25 studies (25/30, 83%) [27-30, 32-37, 39, 41, 43-49, 51-56]. In three others (3/30, 10%), group allocation was assessed as high risk, based on the experiment set-up, since comparison groups only had one or a non-specified amount of animals [31, 38, 40]. In two recent studies, either the animals or both kidneys of one animal (paired study design) were randomly assigned to different experimental groups [42, 50]. Baseline characteristics (animal strain, sex, weight, or diet) were mostly well described. In five studies (5/30, 17%), the different groups did not have similar baseline characteristics or analyses were not adjusted for confounders [35, 38, 48, 51, 55]. Performance bias was rated as high in three studies [31, 34, 40], low in one study [42], and unclear in all others (26/30, 87%) [27-30, 32, 33, 35-39, 41, 43-55]. Random housing of animals was not performed in two studies (2/30, 7%) [31, 40] and was not reported in the remaining studies [27-30, 32-39, 41-56]. Due to the nature of the paired study set-up used by Patel *et al.*, animal caregivers were automatically blinded [42]. The risk of detection bias was unclear in most of the studies (23/30, 77%) [27-30, 33, 35-37, 39, 41, 43-49, 51-56]. A low risk was found in the two recent studies [42, 50] whereas five older studies [31, 32, 34, 38, 40] were deemed to have high risk because outcomes were not assessed randomly (5/30, 17%) [31, 32, 34, 38, 40] or the outcome assessor was not blinded (2/30, 7%) [31, 40]. Risk of attrition bias was high in 18 studies (18/30, 60%) [27-30, 35, 37, 38, 40, 43, 45-49, 51, 53, 55, 56], low in two studies (2/30, 7%) [33, 36] and unclear in the others (10/30, 33%) [31, 32, 34, 39, 41, 42, 44, 50, 52, 54]. In 11/30 (37%) articles, outcomes were not reported selectively, reducing the risk of reporting bias [31, 32, 34, 36, 39, 41, 42, 44, 50, 52, 54]. Three (3/30, 10%) studies were evaluated as high risk for reporting bias [37, 38, 40]. The risk on another potential bias was

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assessed as high in eight articles (27%) [28, 29, 34, 37, 45, 46, 53, 55] and low in one study [42], mainly based on evaluation of potential analysis errors, design-specific risks of bias, unclear reporting on exclusions due to technical failures or no information on possibly received funding. The risk of potential bias was unclear in all 21 other articles [27, 30-32, 34-36, 38-41, 43, 44, 47-54].

In the hypothermic perfusion group (Table S3, S4), two recent studies had low risk of bias [41, 42] whereas three older studies were assessed to have a high risk of selection, attrition, and reporting bias [37, 38, 48]. In the normothermic perfusion group (Table S3, S4), risk of bias was mainly unclear. Five studies (5/23, 22%) showed low risk of bias, when assessable [36, 39, 45, 50, 52]. Of these, two were recently published [39, 50]. However, in five articles risk of bias was assessed high on different domains [31, 35, 40, 51, 55].

Hypothermic kidney perfusion

Hypothermic isolated kidney perfusion set-up

All six studies reporting on the use of metabolite tracers during *ex situ* hypothermic kidney perfusion investigated metabolism in large animals (Table 1) [37, 38, 41, 42, 44, 48]. Between 1974 and 1979, dog kidneys were used for research with temperatures ranging from 5 to 12°C. Length of perfusion ranged from 18 hours [42] to 6 days [37, 38, 44]. Lundstam *et al.* set the perfusion target pressure at 60 mmHg. No (ischemic) damage to the kidneys was mentioned. In contrast, two recent studies exposed pig kidneys to 15 min of warm ischemia and then deliberately cold stored them for 120 minutes before hypothermic perfusion for 18-24h at a target pressure of 30 mmHg [41, 42].

Acellular perfusates were used in all studies without the use of oxygen carriers (Table 2). In four papers, oxygenation of the perfusate was mentioned [37, 42, 44]. Two dog studies mention the adding of a mixture of 66% N_2, 33% O_2 and 1% CO_2 to the oxygenator [37, 44]. Lundstam *et al.* oxygenated the perfusate with a mixture of 99% O_2 and 1% CO_2 [38]. Although Skrede *et al.* mentioned the use of an oxygenator, no further details were given [48]. In pigs, Patel *et al.* investigated whether adding

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oxygen (95%) versus exposure to air (21%) via a membrane oxygenator would result in metabolic changes [38, 48]. In the study of Nath *et al.* no additional oxygen was added to the circuit [41]. In both pig studies, Kidney Perfusion Solution-1 [41, 42], a specific hypothermic perfusion solution designed for preservation of human kidneys for transplantation, or human serum albumin [37, 38, 44, 48] were used as prime solution (Table 3). None of these studies added extra amino acids to the perfusate. Two studies added a vasodilator (papaverine) [37, 44].

Patel *et al.* perfused human donor kidneys before transplantation with Kidney Perfusion Solution-1 with labelled glucose using a LifePort Kidney Transporter at a perfusion pressure of 30 mmHg [57]. The conference abstract does not mention the use of active oxygenation of the perfusate.

Active metabolic pathways in hypothermically perfused kidney

Studies were categorized as either studying carbohydrate (4/6, 66%) [37, 41, 42, 44], amino acid (1/6, 17%) [38], and lipid (2/6, 33%) [37, 48] metabolism (Table 4). Lundstam *et al.* combined research on carbohydrate and lipid metabolism in one study [37, 48]. Both radioactive (4/6, 67%) [37, 38, 44, 48] and non-radioactive, stable isotope tracers (2/6, 33%) [41, 42] were used, all containing carbon labelling.

Carbohydrate metabolism: Petterson *et al.* found that radioactively labelled glucose was mainly converted into lactate whereas only small amounts of radioactivity were found in glycogen and CO_2 [44]. These findings were confirmed by Lundstam *et al.* who added radioactively labelled glucose and lactate to separate experiments of hypothermic perfusion of dog kidneys [37]. The studies were designed to examine the role of metabolic inhibition at the level of pyruvate dehydrogenase on the production rate of lactate from glucose to obtain indirect information on the sufficiency of the oxygen supply – 33% of oxygen was given via membrane oxygenator – in this perfusion condition [37]. They found that metabolic blockade of glucose catabolism by multiple mechanisms was at least a contributive factor for lactate production during hypothermic perfusion [37].

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Recently, Nath *et al.* piloted the use of 2D-NMR spectroscopy to analyze the fate of non-radioactively labelled glucose in the perfusate [41]. After 6h of perfusion, fully labelled lactate, alanine, and acetate were identified confirming active metabolism during hypothermic perfusion. Lactate and alanine concentrations and labelling percentages increased further during perfusion. In cortex biopsies at 24h of perfusion, the same compounds were identified together with newly formed glutamate, where C-atoms at place 4 and 5 were labelled (indicative for citric acid cycle activity). Patel *et al.* went further and performed a paired study where pig kidneys exposed to warm ischemia were either assigned to oxygenated or aerated hypothermic perfusion [42]. Using the same tracer and analyzing technique as Nath *et al.*, they observed lower fully labelled alanine and lactate concentrations in oxygenated compared to aerated perfusion, showing a higher degree of aerobic metabolism with higher oxygen levels. In cortex samples, more 4-5-labelled glutamate was present in oxygenated perfusion [41]. Also, two labelled forms of citrate, succinate and malate (intermediates of the citric acid cycle) were identified. In a follow-up study, identified only as a Conference Abstract, Patel *et al.* supplemented the perfusate of 14 human kidneys preserved by hypothermic perfusion with ^{13}C-glucose and analyzed perfusate samples by 1D and 2D-NMR spectroscopy, in addition to gas chromatography-mass spectrometry [57]. Kidneys donated after circulatory death had higher perfusate levels of ^{13}C-alanine and ^{13}C-lactate compared to kidneys donated after brain death, showing that these kidneys are reliant on anaerobic metabolism early during perfusion.

Amino acid metabolism: Lundstam *et al.* showed incorporation of radioactively labelled threonine and leucine in dog kidney cortex proteins during hypothermic perfusion [38].

Free fatty acid metabolism: Two dog studies added radioactively labelled short- (acetate, caprylic acid) or long-chain fatty acids (palmitate, linoleate, oleate, myristic acid) to their perfusates [37, 48]. Adding labelled acetate [37] or caprylic acid [44] resulted in signs of decarboxylation (appearance of labelled CO_2), and incorporation of labelled carbons in glucose and to a lesser extent into lactate. Palmitate, on the other hand, was oxidized to CO_2 at a very low rate (0.4%) as found by both studies.

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Exchange happened mainly with phospholipid fatty acids and no conversion of lineolate to arachidonic acid could be demonstrated. Linoleic, palmitic, and myristic acid were mainly converted into phospholipids and at a lower rate into triglycerides whereas caprylic acid was not incorporated [37].

Normothermic kidney perfusion

Normothermic isolated kidney perfusion set-up

All experiments were set-up in rodent models [27-36, 40, 43, 45-47, 49-55] except for one recent pig study [39] (Table 1). Although most papers describe tracer studies in healthy kidneys, some investigators used a (chronic) disease model where the kidney was injured *in vivo* before the start of the experiment. Hsueh *et al.* studied hydronephrotic kidneys (bilateral ligation of the ureters 3 days in advance) [55]. Funahashi *et al.* induced diabetes by administration of streptozotocin [32]. Summerfield *et al.* ligated the rat's bile duct to induce chronic bile duct obstruction [49]. van Erp *et al.* induced brain death in rats (inflation Fogarty catheter in the epidural space) to evaluate the effect of organ donation after brain death on kidney metabolism [50].

Although three studies described *in situ* perfusion [52-54] most kidneys were retrieved and connected to an *ex situ* perfusion device. In rats, the right renal artery was cannulated via the mesenteric artery, avoiding interruption of oxygen/nutrient supply (ischemia) in the majority of studies (17/23, 74%) [27-31, 33-36, 40, 43, 45-47, 49, 52, 54], as described by Nishiitsutsuji-Uwo *et al.* [58] In pigs, kidneys were retrieved and prepared for cannulation *ex situ* (mimicking clinical practice of organ retrieval and perfusion). As this takes time, kidneys were cooled down after retrieval resulting in a short cold ischemia period (±32 minutes) before normothermic perfusion [39]. Reported kidney perfusion times ranged from 30 to 480 minutes (Table 2).

Normothermic perfusion was either pressure (13/23, 57%) [27, 29-34, 36, 46, 47, 50, 52, 53] or flow driven (4/23, 17%) [39, 40, 49, 54] or not mentioned (6/23, 26%) [28, 35, 43, 45, 51, 55] (Table 2). When pressure driven, target arterial pressure was uniformly set at 100 mmHg [27, 30-34, 36, 46, 47,

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50, 52, 53], except in one study (120 mmHg) [29]. When flow-driven target perfusion was used, flows varied from 30 to 40 ml/min/g in rats [40, 49] with one study using lower flow rates (12-15 ml/min/g) [54] and 170 ml/min in pigs [39] (Table 2). Vasodilators (bradykinin [55], prostacyclin [51], verapamil [39]) were added to the circuit in three studies (3/23, 13%) [39, 43, 55] (Table 3).

The main perfusate component (prime solution) were crystalloid based solutions containing glucose and various amino acids (Krebs-Henseleit (12/23, 52%) [27, 31-34, 40, 45-47, 52, 53, 55], Krebs-Ringer (8/23, 35%) [28-30, 35, 36, 43, 49, 54], Tyrode's solution (1/23, 4%) [51], or William's Medium E GlutaMAX (1/23, 4%) [50]; composition of the solutions is summarized in Table S5), sometimes supplemented with albumin to increase oncotic pressure (Table 3). Extra glucose or amino acids were added in respectively 15 (65%) and 10 (43%) studies, depending on the composition of the prime solution (Table 3). The use of other additives like antibiotics, inulin, insulin, and other metabolites was described in 67% of the studies. Only one study, with pig kidneys, used whole blood diluted with Ringer's lactate [39]. Perfusate supplementation with 95% O_2 and 5% CO_2 was provided in the majority of studies (19/23, 83%) [27, 29-34, 36, 39, 40, 44-47, 49, 50, 52, 53, 55] and not mentioned in four (17%) [28, 35, 51, 54]. Remarkably, and despite high oxygen delivery at normothermic temperatures, an oxygen carrier (red blood cells/perfluorocarbons) was only used in two studies (one with rat, the other with pig kidneys) [39, 47].

Active metabolic pathways in normothermically perfused kidney

Studies were categorized as either studying carbohydrate (4/23, 17%) [29, 30, 39, 50], amino acid (5/23, 22%) [27, 32, 40, 43, 47], metabolism of other molecules (4/23, 17%) [33, 34], and synthesis of macromolecules [51-53, 55] (Table 5). Both radioactive (20/23, 87%) [27-30, 32-36, 40, 43, 45-47, 49, 51-55] and non-radioactive, stable isotope tracers (3/23, 13%) [31, 39, 50] were used, containing either carbon, nitrogen, hydrogen, sulphur, or iodine labelling.

Carbohydrate metabolism: Cohen *et al.* showed that a stepwise increase of lactate concentration (from 0 to 10 mM) resulted in increased lactate utilization but plateauing of lactate oxidation rates

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when concentrations reached 4.2 mM in the absence of other substrates, suggesting gluconeogenesis from lactate in rat kidneys [29]. In a second study, Cohen *et al.* showed that perfusate composition effects lactate metabolism as a lower net utilization and decarboxylation of lactate was seen when the kidney was perfused with a substrate free albumin compared to bovine serum albumin [30]. Mariager *et al.* showed that pyruvate metabolism differs in *ex situ* versus *in vivo* conditions in pig kidneys perfused with a red blood cell based perfusate [39]. A recent rat study showed that pathological conditions influence glucose metabolism [50]. Glucose oxidation was significantly lower in brain dead animals versus sham when glucose hydrogens were radioactively labelled.

Amino acid metabolism: Two studies investigated the role of the kidney in arginine metabolism [32, 43]. Both added radioactive [^{14}C]-citrulline to the perfusate and found conversion to arginine and guanidinoacetate. Perez *et al.* found label of guanidino-^{14}C-citrulline in urea, creatine, and guanidine derivatives in healthy kidneys [43]. Funahashi *et al.* found that conversion to guanidinoacetate was impaired in kidneys from diabetic rats and did not improve by insulin treatment [32].

Serine metabolism was studied by adding ^{14}C-aspartate to the perfusate [47]. Radioactively labelled aspartate was incorporated into serine and glucose, showing that the (non) phosphorylated triose pathway is a major pathway for serine synthesis. Aspartate label was also found in tissue malate and fumarate.

Bogusky *et al.* added two different forms of radioactively labelled glutamine [27]. One in which nitrogen of the amido and one in which the nitrogen from the amino group was labelled. They found that lowering perfusate pH increases the rate of glutamine deamidation.

Metabolism of branched-chain amino acids (leucine, isoleucine, and valine) to their 2-oxo acids was studied by Miller *et al*. [40]. Oxo-acids from branched chain amino-acids were released into the perfusate. Valine transamination increased linearly while oxidation increased exponentially. Adding the 2-oxo acid of valine resulted in increased oxidation and formation of valine while concentrations of isoleucine and leucine decreased and levels of their oxo-acids increased.

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Other metabolism: Rat studies with radioactively labelled A- or B-chain insulin suggest insulin degradation by the kidney has similar cellular mechanisms to those in the liver [55]. The release of partially degraded insulin into the perfusate also suggests that either some degradation occurs on the plasma membrane without requiring internalization or that partially degraded insulin is released from an intracellular site [55].

Active metabolization of carnitine and acylcarnitines in the kidney was shown. α-keto-acids stimulated acetylcarnitine, isovalerylcarnitine, and isobutyrylcarnitine production from methyl-^{3}H-carnitine whereas adding propionate inhibited their production [33]. Furthermore, adding [^{14}C]-labelled keto-acids resulted in formation of labelled 3-hydroxyisobutyrate, 3-hydroxyvalerate, 2-methyl-3-hydroxybutryate, branched chain amino-acids, branched-chain acylcarnitines, and lactate [34].

Urinary bile acid monosulphate synthesis in rats was shown by Summerfield *et al.* and this was not influenced by bile-duct ligation [49]. Perfusate composition influenced bile acid excretion with less urinary excretion in the presence of albumin compared to a protein-free substrate.

Biosynthesis of macromolecules: Prostaglandin production was studied in rabbits [51, 55]. Hsueh *et al.* showed efficient incorporation of radioactively labelled arachidonic acid into tissue lipids (mainly phospholipids) [55]. Labelling was found pre-dominantly in the cortical region and was confined to vascular tissue (compared to more diffuse labelling) when perfusing with a protein free compared to an albumin containing perfusate. This incorporation occurred in both healthy and hydronephrotic kidneys. Labelled arachidonic acid was converted into a mixture of prostaglandin I_2, F_2, and E_2 whereas stimulation of release of endogenous arachidonic acid (by ischemia or bradykinine) resulted only in prostaglandin E_2 production. Wong *et al.* identified two major products of prostacyclin I_2 beta-oxidation in the kidney [51].

Thromboxane B_2 (TXB_2) was shown to be metabolized by the kidney through beta-oxidation (2,3-dinor-TXB_2, 2,3-dinor-TXB_1, and 2,3,4,5-tetranor-TXB_1), with only little excretion in the urine in healthy kidneys [52, 53].

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Two studies investigated cholesterol synthesis using labelled mevalonate [28, 36]. Brunengraber *et al.* showed that 46% of R[3-^{14}C]-mevalonate was incorporated into lipids (saponifiable, digitonin-precipitable sterols, and squalene and prenols) while 22% was secreted in the urine [28]. Kopito *et al.* showed a potential role of the mevalonate shunt pathway in long-term regulation of cholesterol synthesis in the kidney as 17% of mevalonate entered this pathway instead of the cholesterol synthesis pathway [36].

Incorporation of [^{35}S] into proteoglycan was shown to be impaired in hyperglycemia. Kanwar *et al.* showed 30-40% less [^{35}S]-sulphate incorporation in the glomerular extracellular matrices in a diabetic kidney (streptozotocin induced diabetes) compared to control [54]. Furthermore, [^{35}S]-methionine was incorporated into type IV collagen, laminin, or in the core peptide of heparan sulfate-proteoglycans when a hyperglycemic state was mimicked during perfusion by adding mannose, galactose, or glucose to the perfusate [35].

Vitamin D_3 metabolites containing a 25-hydroxyl group was found to stimulate ^{3}H-24R,25$(OH)_2D_3$ production [45]. Calcioic and cholacalcioic acid were identified as end-products of 25-OHD_3 metabolism through the C-24 oxidation pathway. As their production enhanced in vitamin D_3 intoxicated rats, C-24 oxidation can play a protective role by inactivating 25$(OH)D_3$ [46].

Discussion

This systematic review assessed the models and conditions in which tracer studies have been used to investigate the metabolic behavior of kidneys during hypothermic or normothermic kidney perfusion and summarized the findings of these studies.

Studies fell in one of two eras. The first era is historical, with articles published between 1974 and 1992. These studies generally focused on unraveling kidney metabolism in healthy conditions in both hypothermic and normothermic perfusion. Some investigated pathophysiology of chronic disease

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models (diabetes, hydronephrosis). Regarded by today's quality standards, the risk of bias in these studies is high. The second era is recent, with papers identified between 2006 and 2020. These studies explored the feasibility of adding primarily non-radioactive, stable isotope labelled metabolites to assess kidney viability in the setting of kidney transplantation. Risk of bias in these studies was lower than those of the historic era, though many items remained unclear, pointing towards the need for more complete reporting of study methodology with particular emphasis on risk of bias.

The tracer studies identified here show active metabolism of carbohydrate, amino acid, and lipid metabolism in hypothermic conditions with less aerobic metabolism in conditions of lower oxygenation. While studies have shown the need for high partial oxygen tensions to support ATP production during hypothermic kidney perfusion, only one study provides information on partial oxygen tensions in the perfusate [59]. The solubility of oxygen in cold temperatures is high and therefore high partial oxygen tensions are easily reached with limited gas flow of 100% oxygen through a membrane oxygenator [60]. This supports the findings of a recent randomized trial showing improved outcomes of kidneys transplanted after preservation by oxygenated hypothermic perfusion compared to those that were preserved by hypothermic perfusion without supplemental oxygenation [14]. Whether these findings are translatable to the setting of kidney preservation is unclear, though the feasibility of the use of tracers in the clinical setting has been shown by Patel *et al.*, who perfused human donor kidneys with non-radioactively labelled glucose during hypothermic perfusion and subsequently transplanted these kidneys [57]. From the Conference Abstract it is unclear whether the kidneys received oxygen during perfusion.

Tracer studies in healthy kidneys perfused at normothermia found evidence of active glucose, amino acid, and metabolism of other large molecules as well as biosynthesis of macromolecules such as prostaglandins, Vitamin D. The aims of the experiments were highly variable with mostly

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heterogenous research questions for each experiment and we cannot draw any overall conclusions on the data presented here. However, the use of tracers allowed researchers to address specific hypotheses.

It is clear from these studies that the metabolism of the normothermically perfused healthy kidney is incompletely understood. Furthermore, we do not understand how this metabolism changes in perturbed conditions, such as ischemia. Both are vital to unravel, firstly to improve our overall knowledge on kidney metabolism and secondly to ensure that the metabolic needs of donor kidneys – that will have been exposed to some form of injury (e.g. brain death, hypoperfusion, ischemia) – are met during preservation. In that light, it is important to recognize that that glucose oxidation was found to be significantly lower in brain dead rats compared to sham [50], underpinning the likeliness that donor kidneys behave differently compared to healthy kidneys.

There are, however, a few important issues to consider. These studies were performed in normothermic conditions making use of an oxygenated crystalloid based perfusate containing glucose and additional nutrients. The variability of the perfusion solution makes direct comparison of the outcomes difficult and it is important to realize that the composition of the perfusion solution might affect or alter metabolism during perfusion, as was shown by Cohen *et al.* and Hsueh *et al.* [29, 30, 55] Furthermore, oxygen carriers (such as red blood cells) were seldomly used. The low solubility of oxygen at atmospheric pressures and normothermic temperatures was counteracted by actively oxygenating the gas mixture (95% O_2, 5% CO_2), however, the flow rate of the gas mixture and the actual partial oxygen tensions, which are critical to assess the results, were rarely mentioned. As oxygen uptake by cells is bound by diffusion gradients between perfusate and cytosol, it is difficult to know whether the oxygen requirements of the cells were met in these models. In the few cases where an oxygen carrier was used, the gas mixture was not adapted, most likely resulting in hyperoxia. This, too, might have changed the normal physiological metabolism. Therefore, any results found in these

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studies, and their translatability to human settings, needs to be interpreted with the necessary caution.

The recent and sudden regained interest in the use of tracer metabolomics in kidney perfusion can be explained by multiple factors.

Firstly, the availability of non-radioactive tracers and advanced analyses methods in the metabolomics field have created the opportunity to not only explore *in vivo* metabolism [61-63] in depth but also to use these to further elucidate the metabolism of the single, isolated organ. Indeed, isolated from the neural, hormonal stimuli and feedback mechanisms of other organs, metabolism of the kidney *ex situ* is likely to be different from that *in vivo*. Indeed, the findings by Mariager *et al.*, show different use of pyruvate by pig kidneys in *ex situ* compared to *in vivo* conditions [39]. Although tracers are powerful tools to follow the metabolism of specific compounds, some consideration needs to be given to the choice of the labelled compound (which needs to be actively metabolised by the organ), the labelled atom (e.g. labelling of nitrogen in an amino acids will allow to follow the nitro group but not the carbon bonds), and the tracer type. Indeed, radioactive tracers are more limited in comparison to the stable isotope tracers as the majority of them are only capable of monitoring one specific enzyme reaction and thus fail, in contrast to stable isotopes, to monitor a broad set of enzymatic reactions at once.

Secondly, hypothermic kidney perfusion has become a recognized kidney preservation platform after large well-designed studies showing improved outcomes with hypothermic perfusion compared to static cold storage [7]. Recently, we showed that supplementing the perfusate with oxygen, to reach high partial oxygen tensions, improves outcomes after transplantation of kidneys donated after circulatory death [14]. In that respect, it is encouraging that the use of tracers has shown active metabolism during hypothermic kidney perfusion of porcine and human kidneys as maintaining aerobic metabolism might be the underlying reason for improved graft preservation.

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Thirdly, the increased use of less-than-ideal deceased donor kidneys for transplantation has heightened the clinical need for viability assessment and organ repair. Classic static cold storage cannot meet these needs and therefore *ex situ* kidney perfusion is increasingly studied. The use of 2D-NMR to detect non-radioactive labelled glucose has been shown feasible in normothermic pig kidney studies [41] and might open a door towards not only a better understanding of kidney metabolism but also potentially predicting viability of injured kidneys before they are transplanted. In that light, it is important to note that none of kidneys were not transplanted in the identified experimental studies.

As with all systematic reviews, it is possible that some relevant articles were not identified or that relevant studies were published after the search. We limited the chance of missing relevant articles by setting up a broad search strategy in collaboration with experienced biomedical reference librarians. Furthermore, references of included articles were searched to identify any articles that might have been missed by in the systematic search.

In conclusion, tracers have been used in the setting of preclinical models of isolated kidney perfusion to investigate how healthy and diseased kidneys metabolize nutrients. It is clear that adding a (non)radioactively labelled compound to kidney perfusion set-ups is feasible and can contribute greatly to unravelling the metabolic behavior. This is vital to further develop kidney perfusion as a platform for organ preservation, viability assessment, and resuscitation. Whether findings, particularly those in normothermic perfusion, are truly translational remains to be shown as most studies were performed in rodents with considerable differences in perfusion solution and oxygenation levels compared to physiological conditions. In that respect well-designed tracer studies, that mimic physiology as closely as possible, in large animal models, human organs not fit for transplantation, or in the setting of a clinical study would be very valuable. In particular, understanding how metabolism changes after exposure to ischemia is essential in the transplantation setting.

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Data Availability Statement: The full data extraction table of this systematic review is accessible via https://doi.org/10.48804/MLBUAS [24].

Conflicts of Interest: I.J. and B.G. are listed as co-inventor of a patent application on methods and applications of analyzing the perfusate of an ex situ perfused kidney (EP 22155190.6). I.J. received speaker fees from XVIVO Perfusion paid to her institution.

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Tables

Table 1. Overview of included studies summarizing species, model, and perfusion temperature.

Study	Species	Model	Temp (°C)	Disease model*
Normothermic perfusion				
Cohen 1975 **[30]**	Rat	Ex situ	38-39	healthy
Summerfield 1976 **[49]**	Rat	Ex situ	38	bile-duct ligation
Cohen 1977 **[29]**	Rat	Ex situ	38	healthy
Perez 1978 **[43]**	Rat	Ex situ	37	N/A
Hsueh 1978 **[55]**	Rabbit	Unclear	37	hydronephrotic
Wong 1978 **[51]**	Rabbit	Ex situ	37	healthy
Brunengraber 1981 **[28]**	Rat	Ex situ	38	healthy
Funahashi 1981 **[32]**	Rat	Ex situ	37	diabetes
Kanwar 1983 **[54]**	Rat	In situ	30-35	diabetes
Reddy 1983 **[45]**	Rat	Ex situ	38	Vit D intoxication
Kopito 1984 **[36]**	Rat	Ex situ	38-40	healthy
Miller 1984 **[40]**	Rat	Ex situ	38-40	healthy
Scaduto 1985 **[47]**	Rat	Ex situ	37	healthy
Hokland 1986 **[33]**	Rat	Ex situ	38-40	healthy
Hokland 1988 **[34]**	Rat	Ex situ	38-40	healthy
Benigni 1989 **[52]**	Rat	In situ	37	healthy
Bogusky 1989 **[27]**	Rat	Ex situ	38-40	healthy
Chiabrando 1989 **[53]**	Rat	In situ	37	healthy
Duckworth 1989 **[31]**	Rat	Ex situ	37	healthy
Kanwar 1992 **[35]**	Rat	Ex situ	N/A	healthy
Reddy 2006 **[46]**	Rat	Ex situ	37	healthy
van Erp 2020 **[50]**	Rat	Ex situ	37	brain death
Mariager 2020**[39]**	Pig	Ex situ	38	cold ischemia
Hypothermic perfusion				
Pettersson 1974 **[44]**	Dog	Ex situ	5-7	healthy
Lundstam 1976 **[37]**	Dog	Ex situ	6-8	healthy
Lundstam 1977 **[38]**	Dog	Ex situ	10	healthy
Skrede 1979 **[48]**	Dog	Ex situ	8-12	healthy
Nath 2016 **[41]**	Pig	Ex situ	4	warm + cold ischemia
Patel 2019 **[42]**	Pig	Ex situ	4	warm + cold ischemia
Perfusion temperature not specified				
Welbourne 1977 **[56]**	Rat	Ex situ	N/A	N/A

*, chronic model in the animal (e.g. diabetes was induced several days before experiment); N/A, not available

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Table 2. Overview of included studies summarizing perfusion settings.

Study	Pressure (mmHg)	Flow (ml/min)	Perfusion length	O_2 carrier	FiO_2 (%)	Gas flow (l/min)	pO_2 (mmHg)
Hypothermic perfusion							
Pettersson 1974 **[44]**	60	N/A	6d	No	33	N/A	N/A
Lundstam 1976 **[37]**	60	N/A	6d	No	33	N/A	N/A
Lundstam 1977 **[38]**	N/A	N/A	6d	No	99	N/A	N/A
Skrede 1979 **[48]**	N/A	N/A	45h	No	N/A	N/A	N/A
Nath 2016 **[41]**	30	N/A	24h	No	N/A	N/A	N/A
Patel 2019 **[42]**	30	N/A	18h	No	95	0.1	150*
Normothermic perfusion							
Cohen 1975 **[30]**	100	N/A	80 min	No	95	N/A	N/A
Summerfield 1976 **[49]**	N/A	32-34	60 min	No	95	N/A	N/A
Cohen 1977 **[29]**	120	N/A	75 min	No	95	0,3	N/A
Perez 1978 **[43]**	N/A	N/A	90 min	No	95	N/A	N/A
Hsueh 1978 **[55]**	N/A	N/A	191	No	95	N/A	N/A
Wong 1978 **[51]**	N/A	N/A	N/A	No	N/A	N/A	N/A
Brunengraber 1981 **[28]**	N/A	N/A	65 min	No	N/A	N/A	N/A
Funahashi 1981 **[32]**	100	N/A	30 min	No	95	N/A	N/A
Kanwar 1983 **[54]**	120-150	12-15	7-8h	No	N/A	N/A	N/A
Reddy 1983 **[45]**	N/A	N/A	6h	No	95	N/A	N/A
Kopito 1984 **[36]**	100 +/-5	N/A	65 min	No	95	N/A	N/A
Miller 1984 **[40]**	N/A	30-40	90 min	No	95	N/A	N/A
Scaduto 1985 **[47]**	100	N/A	60 min	Yes	95	N/A	N/A
Hokland 1986 **[33]**	90-100	N/A	30 min	No	95	N/A	N/A
Hokland 1988 **[34]**	100	N/A	40 min	No	95	N/A	N/A
Benigni 1989 **[52]**	95-100	N/A	80 min	No	95	N/A	N/A
Bogusky 1989 **[27]**	100 +/-5	N/A	60 min	No	95	N/A	N/A
Chiabrando 1989 **[53]**	100	N/A	60 min	No	95	N/A	N/A
Duckworth 1989 **[31]**	100	N/A	75 min	No	95	N/A	N/A
Kanwar 1992 **[35]**	N/A	N/A	300 min	No	N/A	N/A	N/A
Reddy 2006 **[46]**	100	N/A	480 min	No	95	N/A	400
van Erp 2020 **[50]**	100	N/A	90 min	No	95	N/A	450
Mariager 2020 **[39]**	N/A	170	120 min	Yes	95	0.5	N/A
Perfusion temperature not specified							
Welbourne 1977 **[56]**	N/A	N/A	60 min	No	95	N/A	N/A

*, 150 mmHg at the start of perfusion, rapidly decreasing to absolute anoxia (11 mmHg) within 90 minutes. FiO_2, oxygen concentration; N/A, not available; O_2; oxygen

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Table 3. Overview of included studies summarizing perfusion settings.

Study	Species	Perfusate	Amino acids	Glucose	Vasodilator
Hypothermic perfusion					
Pettersson 1974 **[44]**	Dog	Albumin	No	Yes	Yes
Lundstam 1976 **[37]**	Dog	Saline (+Albumin)	No	Yes	Yes
Lundstam 1977 **[38]**	Dog	Albumin solution	Yes	Yes	No
Skrede 1979 **[48]**	Dog	Albumin solution	No	No	No
Nath 2016 **[41]**	Pig	Kidney Preservation Solution-1	No	No	No
Patel 2019 **[42]**	Pig	Kidney Preservation Solution-1	No	Yes	No
Normothermic perfusion					
Cohen 1975 **[30]**	Rat	Krebs-Ringer (+Albumin)	No	No	No
Summerfield 1976 **[49]**	Rat	Krebs-Ringer	No	Yes	No
Cohen 1977 **[29]**	Rat	Krebs-Ringer (+Albumin)	No	Yes	No
Perez 1978 **[43]**	Rat	Krebs-Ringer (+Albumin)	Yes	Yes	No
Hsueh 1978 **[55]**	Rabbit	Krebs-Henseleit (+ Albumin)	No	No	Yes
Wong 1978 **[51]**	Rabbit	Tyrode's	No	No	Yes
Brunengraber 1981 **[28]**	Rat	Krebs-Ringer (+Albumin)	No	Yes	No
Funahashi 1981 **[32]**	Rat	Krebs-Henseleit	Yes	No	No
Kanwar 1983 **[54]**	Rat	Krebs-Ringer	N/A	N/A	N/A
Reddy 1983 **[45]**	Rat	Krebs-Henseleit (+Albumin)	No	Yes	No
Kopito 1984 **[36]**	Rat	Krebs-Ringer (+Albumin)	No	Yes	No
Miller 1984 **[40]**	Rat	Krebs-Henseleit (+Albumin)	Yes	Yes	No
Scaduto 1985 **[47]**	Rat	Krebs-Henseleit	No	No	No
Hokland 1986 **[33]**	Rat	Krebs-Henseleit	No	Yes	No
Hokland 1988 **[34]**	Rat	Krebs-Henseleit (+Albumin)	Yes	Yes	No
Benigni 1989 **[52]**	Rat	Krebs-Henseleit (+Albumin)	Yes	Yes	No
Bogusky 1989 **[27]**	Rat	Krebs-Henseleit (+Albumin)	Yes	No	No
Chiabrando 1989 **[53]**	Rat	Krebs-Henseleit (+Albumin)	Yes	Yes	No
Duckworth 1989 **[31]**	Rat	Krebs-Henseleit (+Albumin)	Yes	Yes	No
Kanwar 1992 **[35]**	Rat	Krebs-Ringer	Yes	Yes	No
Reddy 2006 **[46]**	Rat	Krebs-Henseleit (+Albumin)	No	Yes	No
van Erp 2020 **[50]**	Rat	William's Medium E GlutaMAX (+Albumin)	No	No	No
Mariager 2020 **[39]**	Pig	Ringer lactate	Yes	Yes	Yes
Perfusion temperature not specified					
Welbourne 1977 **[56]**	Rat	Krebs Henseleit	Yes	Yes	No

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Table 4. Summary of studies reporting on tracer use during hypothermic kidney perfusion.

Study reference	Metabolic pathway	Tracer*	Findings
Carbohydrate metabolism			
Pettersson 1974 **[44]**	Glucose	**[^{14}C]-glucose**	Glucose was mainly converted into lactate and incorporated to a lesser extent into glycogen or CO_2.
Lundstam 1976 **[37]**	Lactate Glucose Acetate	**[^{14}C]-lactate** **[^{14}C]-glucose** **[^{14}C]-acetate**	Lactate production lower in kidneys perfused with fatty acid-free perfusate versus fatty acid-rich perfusate. Perfusate glucose concentration decreased more rapidly and glucose oxidation was more pronounced with a fatty acid-free perfusate. A metabolic blockade of glucose catabolism by multiple mechanisms is at least a contributive factor for lactate production during hypothermic perfusion. Acetate was utilized by the perfused kidney as an oxidative substrate.
Nath 2016 **[41]**	Glycolysis	^{13}C-glucose	*De novo* metabolism occurs during hypothermic perfusion. Whilst majority of ^{13}C-glucose is metabolized into glycolytic endpoint metabolites (e.g. lactate) there is also presence of non-glycolytic pathway derivatives. Isotopic labelled *ex situ* organ perfusion studies using 2D NMR are feasible and informative.
Patel 2019 **[42]**	Glycolysis Citric acid	^{13}C-glucose	Supplementation of perfusion fluid with high-concentration oxygen (95%) results in a greater degree of aerobic metabolism versus aeration (21%) during hypothermic perfusion.
Amino acid metabolism			
Lundstam 1977 **[38]**	Amino acid	**[^{14}C]-leucine** **[^{14}C]-threonine**	Both leucine and threonine were incorporated into kidney cortex proteins during hypothermic perfusion.
Lipid metabolism			
Lundstam 1976 **[37]**	Acetate	**[^{14}C]-acetate**	Acetate was utilized by the perfused kidney as an oxidative substrate.
Skrede 1979 **[48]**	Fatty acid	**[^{14}C]-palmitate** **[^{14}C]-linoleate**	Palmitic acid was oxidized to CO_2 at a very low rate. No conversion of lineolate to arachidonic acid could be demonstrated.

*, radioactive tracers are depicted in **bold**.

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Table 5. *Summary of studies reporting on tracer use during normothermic kidney perfusion*.

Study reference	Metabolic pathway	Tracer*	Findings
Carbohydrate metabolism			
Cohen 1975 **[30]**	Lactate Gluconeogenesis	**L-(+)-[U-^{14}C]-lactate**	Lactate consumption increased with increasing lactate administration though lactate oxidation plateaued, explained by an increase in gluconeogenesis from lactate.
Cohen 1977 **[29]**	Lactate Glucose	**L-(+)-[U-^{14}C]-lactate** **D[U-^{14}C]-glucose**	Lower net utilization and decarboxylation of lactate with substrate free albumin. Both lactate and glucose administration increased tubular sodium reabsorption.
Mariager 2020 **[39]**	Pyruvate	^{13}C-pyruvate	Reduced pyruvate turnover *ex situ* compared to *in vivo* (MRI/MRS techniques).
van Erp 2020 **[50]**	Glucose oxidation	d-6-^{3}H-glucose	Glucose oxidation significantly lower in brain dead animals versus sham, based on $^{3}H_2O$ production.
Amino acid metabolism			
Perez 1978 **[43]**	Urea cycle	**L-[guanidino ^{14}C]-arginine** **L-[guanidino ^{14}C]-citrulline**	Citrulline converted to arginine and guanidinoacetate. Guanidino ^{14}C-citrulline labelling also found in urea, creatine, and other guanidine derivatives.
Funahashi 1981 **[32]**	Arginine	**[^{14}C]-arginine** **L-[carbamoyl-^{14}C]-citrulline**	Citrulline converted to arginine and guanidinoacetate. Citrulline conversion to guanidinoacetate was impaired in kidneys from diabetic rats and did not improve by insulin treatment.
Miller 1984 **[40]**	Valine Oxo acid	**L-[1-^{14}C]-valine** **3-methyl-2-oxo[1-^{14}C]-butanoate**	Oxo-acids from branched chain amino-acids were released into the perfusate. Valine transamination increased linearly while oxidation increased exponentially. Adding the 2-oxo acid of valine resulted in increased oxidation and formation of valine while concentrations of isoleucine and leucine decreased and levels of their oxo-acids increased.
Scaduto Jr 1985 **[47]**	Serine Gluconeogenesis	**[^{14}C]-aspartate** **[^{14}C]-glycerol**	Labelled aspartate was incorporated into serine and glucose, showing that the (non) phosphorylated triose pathway is a major pathway for serine synthesis.

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Study reference	Metabolic pathway	Tracer*	Findings
Bogusky 1989 **[27]**	Ammonia	**[amido-^{15}N]-glutamine** **[amino-^{15}N]-glutamine**	Regulation of glutamine deamidation is an important controlling step in ammonia formation during acute metabolic acidosis in kidney as lower perfusate pH increases glutamine deamidation rates.
Other metabolism			
Hokland 1986[31, 49] **[33]**	Carnitine	**[methyl-^{3}H]-carnitine**	Perfusion with labelled carnitine and branched chain α keto-acids resulted in excretion of newly formed branched-chain acylcarnitines.
Hokland 1988 **[34]**	Carnitine	**α-keto[U^{14}C]-isovalerate** **α-keto[U^{14}C]-isocaproate** **α-keto[U-^{14}C]-ß-methylvalerate**	Branched-chain hydroxy acids, branched-chain amino acids and branched-chain acylcarnitines and lacate are formed when labelled α keto-acids were added to the perfusate.
Duckworth 1989 **[31]**	Insulin	^{125}iodo(A14)-insulin ^{125}iodo(B26)-insulin	Major insulin products found in the perfusate consist of an intact A-chain and cleaved B-chain and differed from intracellularly found products.
Summerfield 1976 **[49]**	Bile acids	**[24-^{14}C]-chenodeoxycholate** **[24-^{14}C]-litocholate** **[$H_2{}^{35}SO_4$]**	Perfusion with a protein-free perfusate resulted in urinary excretion of lithocholic and chenodeoxycholic acid (3%) and their principal polar metabolites litocholate 3-sulphate and chenodeoxycholate-7-sulphate respectively. Cholestasis did not enhance conversion.
Biosynthesis of macromolecules			
Hsueh 1978 **[55]**	Prostaglandin	**[^{14}C]-AA** **[^{14}C]-PG**	Efficient incorporation of arachidonic acid into tissue lipids (mainly phospholipids). Added arachidonic acid was converted into a mixture of prostaglandin I_2, F_2 and E_2 whereas stimulated release of arachidonic acid from tissues resulted in formation of prostaglandin E_2.
Wong 1978 **[51]**	Prostaglandin	**[9-^{3}H]-PGI2**	Prostaglandin I_2 metabolized into 7,9-dihydroxy-4,13-diketo-dinor-PGF1a (25%) and dinor-6-keto-PGF1a (10%).

This is the version of the paper as accepted by "Artifical Organs", doi: 10.1111/aor.14355

Study reference	Metabolic pathway	Tracer*	Findings
Benigni 1989 **[52]**	Thromboxane	**[^{3}H]-TXB$_2$**	Only 1% of radioactivity was found in urine. Only low levels of TXB$_2$, 2,3-dinor-TXB$_2$, and 11-dehydroxy-TXB$_2$ were excreted in urine (1%).
Chiabrando 1989 **[53]**	Thromboxane	**[^{2}H]-TXB$_2$-d8**	Major metabolites of thromboxane (TX) B2 were identified.
Brunengraber 1981 **[28]**	Cholesterol synthesis	**R[3-^{14}C]-mevalonate** **S[5-^{14}C]-mevalonate**	Sterol synthesis in the kidney appears to be controlled, at least in part, by the level of circulating R-mevalonate.
Kopito 1984 **[36]**	Cholesterol synthesis	**[5-^{14}C]-mevalonate** **[4,5-^{14}C]-mevalonate** **[5-^{3}H] mevalonate**	Mevalonate (17%) entered the mevalonate shunt pathway suggesting a potential role of this pathway in long-term regulation of cholesterol synthesis in the kidney.
Kanwar 1983 **[54]**	Proteoglycan	**[^{35}S]-sulphate**	[^{35}S]-sulphate incorporation into glomerular extracellular matrices was 30-40% less in diabetic kidneys compared to control.
Kanwar 1992 **[35]**	Proteoglycan	**[^{35}S]-sulphate** **[^{35}S]-methionine**	[^{35}S]-methionine was incorporated into type IV collagen, laminin, or in the core peptide of heparan sulfate-proteoglycans when a hyperglycemic state was mimicked during perfusion by adding mannose, galactose, or glucose to the perfusate.
Reddy 1983 **[45]**	Vitamine D	**[^{3}H]-25(OH)D$_3$**	25-hydroxylated vitamin D$_3$ metabolites stimulated ^{3}H-24R,25(OH)$_2$D$_3$ production, where analogues without hydroxylgroups did not.
Reddy 2006 **[46]**	Vitamine D	**[$^{1,2\text{-}3}$H]-25(OH)D$_3$**	Both calcioic and cholacacioic acids are end products of 25(OH)D$_3$ metabolism through the C-24 oxidation pathway. Increased production from 25OHD$_3$ in vitamin D3 intoxicated rats indicate a protective role for C-24 oxidation.

*, radioactive tracers are depicted in **bold**.

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Figure legends

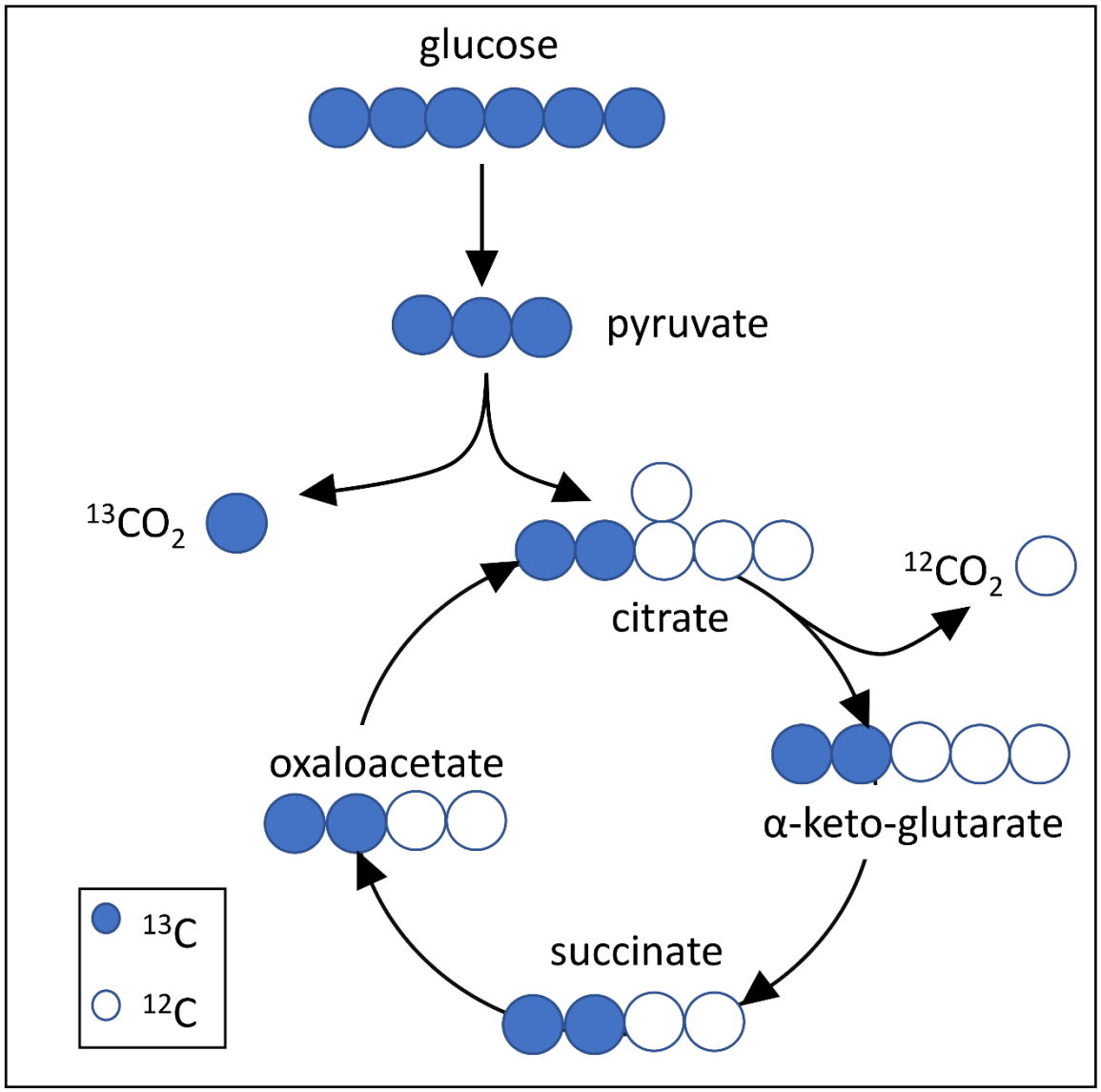

Figure 1. Schematic representation of labelled compounds. If aerobic metabolism is active, a fully labelled glucose molecule would give rise to a fully labelled pyruvate molecule that enters the citric acid cycle. The labelled carbon atoms are incorporated into citrate and downstream, allowing identification of other active pathways.

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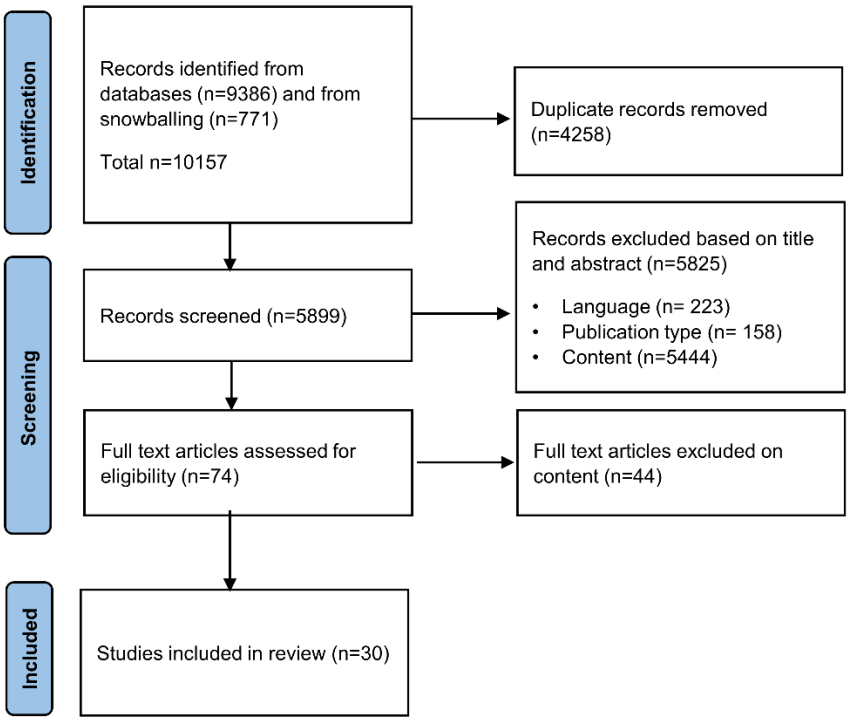

Figure 2. Study flow chart.

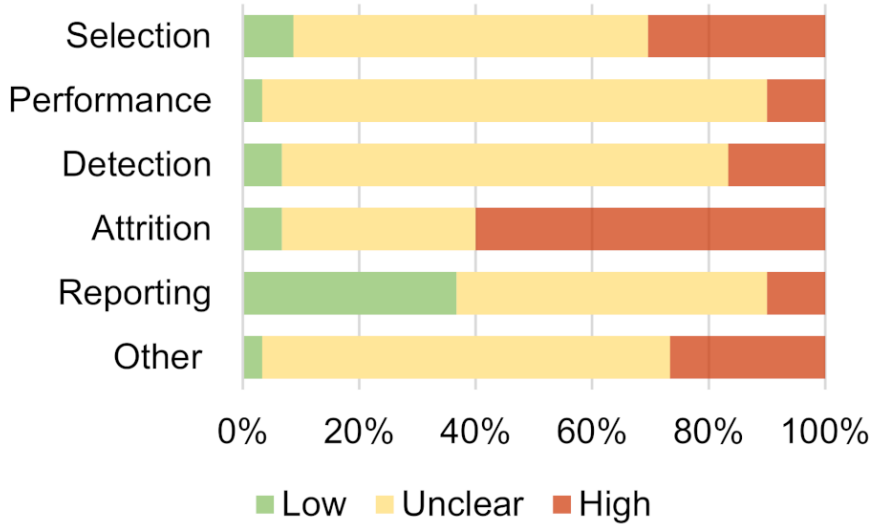

Figure 3. Risk of bias assessment in 30 studies identified, using SYRCLEs tool [26].

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Supplementary Materials:

Table S1: Search string in databases Pubmed, Embase, Web of science, and Cochrane

Table S2: Inclusion and exclusion criteria.

Table S3: Detailed Risk of Bias Assessment using SYRCLE's tool for articles

Table S4: SYRCLE's signaling questions for bias assessment

Table S5: Composition of the media forming the basis for the perfusion solution used in the studies that were identified

Figure S1: Flow chart of systematic search for abstracts reporting on use of tracers in human kidneys

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