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Normothermic machine perfusion (NMP) is emerging as a novel preservation strategy. During NMP, the organ is maintained in a metabolically active state that may not only provide superior organ preservation, but that also facilitates viability testing before transplantation, and *ex situ* resuscitation of marginal kidney grafts. Although the prevailing perfusion protocols for renal NMP are refined from initial pioneering studies concerning short periods of NMP, it could be argued that these protocols are not optimally tailored to address the putatively compromised metabolic plasticity of marginal donor grafts (i.e., in the context of viability testing and/or preservation), or to meet the metabolic prerequisites associated with prolonged perfusions and the required anabolic state in the context of organ regeneration. Herein, we provide a theoretical framework for the metabolic requirements for renal NMP. Aspects are discussed along the lines of carbohydrates, fatty acids, amino acids, and micronutrients required for optimal NMP of an isolated kidney. In addition, considerations for monitoring aspects of metabolic status during NMP are discussed.

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KEYWORDS: kidney transplantation; machine perfusion; metabolism; normothermic; organ preservation

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The key challenge in organ transplantation is the global shortage of donor organs. Because of the pressing demands, most transplant centers are progressively embracing organs from older and higher-risk donors. At the same time, many grafts are discarded because of a perceived risk that these organs may not function, or function suboptimally after transplantation.[1] One strategy to increase graft utility and improve transplant outcomes is the implementation of more objective quality assessment tools by *ex situ* perfusion of donor organs, thereby creating a window for functional testing and viability enhancement of the perfused graft, the 4Rs: resuscitation,[2] repair,[3] rejuvenation,[4] and regeneration.[5] Multiple trials have shown that *ex situ* hypothermic machine perfusion for kidney grafts is feasible and safe, and improves clinical outcomes.[6–8] An obvious next step was the introduction of (sub)normothermic machine perfusion (NMP).[9] Although the feasibility of NMP has been proved in several trials,[10,11] current kidney NMP protocols reflect pioneering proof-of-concept studies, studies that primarily concerned short periods of NMP. The specific metabolic prerequisites may, to some extent, vary with the specific aims of the perfusion; the 4 Rs. Shorter perfusion that aims at resuscitation, rejuvenation, and functional assessment should provide the metabolites that optimally sustain metabolic flexibility. Longer perfusions that aim at repair and rejuvenation come with the additional need for anabolic factors, such as essential amino acids and vitamins. Current protocols are all based on continuous perfusion with red blood cells or an alternate oxygen carrier, an isotonic or/and colloid solution, such as albumin, and glucose and amino acids[2–5,10–13] as energy source.[14] A detailed overview of the published protocols (including the perfusate composition) is provided in Table 1.[2,3,9,10]

Based on the observation that all NMP studies of human kidneys so far report accruing lactate perfusate levels,[3–5,12,13] it could be argued that a physiological metabolic state is not established under the current NMP conditions.[15,16] Although this phenomenon may reflect use of discarded kidneys, lactate accumulation is also observed during NMP of kidneys that were accepted for transplantation.[3] Hence, current NMP

perfusion protocols do not yet entail the optimal conditions required for induction of a physiological metabolic state in viable grafts, let alone that the protocols mimic the optimal conditions for viability testing or prolonged perfusions required for resuscitation and/or repair of so-called "marginal" kidney grafts.

The kidney is a metabolically highly active organ, with a per mass energy requirement that is similar to the heart,[17] and has specific substrate preferences and metabolic functions. The latter aspects are clearly illustrated by the kidney-specific patterns of metabolite uptake and release, shown by organ-specific arteriovenous concentration differences,[18] and by its crucial role in body lactate disposal.[15,19] Moreover, the kidney's functional diversity translates into heterogeneous metabolic profiles with distinct substrate preferences for each of its specialized functional subunits. As a consequence, an optimal NMP protocol should address the metabolic requirements of the kidney and compensate the absence of the body's homeostatic system that would normally replenish nutrients and dispose of waste products.

NMP for viability testing or recovery of so-called "marginal" kidney grafts may come with even more demanding requirements. These "marginal" kidney grafts constitute a heterogeneous group that include grafts from older donors, grafts from perceived higher-risk donors, and/or grafts that have sustained considerable procurement stress (such as prolonged ischemia). These conditions all associate with an impaired resilience,[20] as such "marginal" kidney grafts may present with an impaired metabolic plasticity.[21,22] Consequently, failure to meet the metabolic requirements of these compromised organs may lead to unjustified conclusions with respect to their viability. Similarly, better tailored perfusion protocols are likely required to support an anabolic state and to meet the metabolic prerequisites for prolonged NMP aimed on *ex situ* graft regeneration.

The focus of this review is to provide a theoretical framework of the metabolic aspects of renal NMP (i.e., how to provide optimal metabolic support during graft perfusion). Conclusions from the review may (partially) translate to other organs. Yet, profound organ-specific differences exist with regard to substrate preference.[18] An evaluation of organ-specific differences (such as long-chain fatty acids as the preferred metabolic substrate of the myocardium[23]) was considered beyond the scope of the review. Similarly, aspects of oxygen delivery were considered beyond the scope of the review. This review is structured along the 3 main metabolic clusters: carbohydrates, fatty acids, and amino acids, followed by considerations concerning the provision of micronutrients. Finally, a practical overview of the options for monitoring metabolic homeostasis in the context of NMP is provided. Reference data with regard to aspects of the metabolic physiology of the kidney largely rely on human (living donors) and porcine studies that applied arteriovenous blood sampling over the kidney.[16,18]

Carbohydrates

In general and especially for the kidney, current NMP protocols mainly rely on glucose as metabolic substrate for the perfused graft. However, absent uptake or release of glucose from the kidney, as determined by arteriovenous measurements in the human kidney, could imply minimal renal glucose catabolism (Figure 1[16]). This gross observation ignores the particular complex and spatially diverse organization of renal carbohydrate metabolism, with some areas relying on glycolysis and others actively involved in gluconeogenesis.[15,24,25] This diversity follows the functional heterogeneity of the kidney with broad regional variations in cellular metabolic rates[26] and profound differences in local oxygen tension. Cortical glomeruli represent well-oxygenated vascular structures that principally function as "passive" filters. The medullary tubules, on the other hand, represent a series of highly active, metabolically demanding pumps.[27,28] However, as an inevitable consequence of the countercurrent concentration mechanism of the loops of Henle, aspects of the deeper medulla are exposed to profound hypoxia.[29] Hence, cells in this area are obligatory glycolytic (lactate producing),[30] and reported as relatively resistant to anoxia[31] (Figure 2[25]).

Absent lactate release, or even net lactate uptake from the circulation under physiologic conditions[15,16,18] implies that the lactate formed in the deeper medulla is efficiently cleared within the organ.[32] Recent studies identified the proximal tubules as the primary site of lactate disposal.[15]

Although the renal gluconeogenetic capacity may imply glucose independence, it is important to point out that gluconeogenesis is an anabolic process, and thus imposes an avoidable energy burden to the graft.[33] In addition, inadequate availability of glucose may profoundly interfere with the graft's metabolic plasticity (i.e., the physiologic ability to switch between different metabolic substrates to maintain metabolic competence). As a consequence, provision of an adequate glucose supply is a critical requisite for NMP. In this context, it is important to consider that supraphysiological glucose concentrations will impose an avoidable metabolic burden on proximal convoluted tubules (local burden) within a perfused kidney because blood (perfusate) glucose is filtered into the glomerular ultrafiltrate, and subsequently actively reabsorbed in the proximal convoluted tubule. This process of glucose reabsorption is among the primary energy demanding processes in the kidney.[34] Consequently, from the perspective of minimizing energy requirements during NMP, perfusate physiologic glucose concentration of 3.5 to 5.5 mmol/L should be maintained. It could be speculated that pharmaceutical interference with the active glucose transporters, such as sodium-glucose cotransporter 2 inhibitor, could be advantageous in the context of NMP as it minimizes the energy requisite/burden associated with glucose reabsorption by the distal tubules.[35,36]

A yet unresolved question is whether insulin should also be provided during NMP. Glucose entry is controlled by the family of glucose transporters (GLUTs), most of which are

Table 1 | Overview of the hardware (perfusion control mode, pressure, and gas supply), the perfusate composition, and supplements provided during NMP of the kidney for the different protocols[2–5,10–13]

Perfusate compositions	Hosgood *et al.* (2013, 2017)	Weissenbacher *et al.* (2018)	Kabagambe *et al.* (2018)	Aburawi *et al.* (2019)	Hameed *et al.* (2019)	Minor *et al.* (2020)	Rijkse *et al.* (2021)	Arykbaeva *et al.* (NCT04693325)
Perfusion control mode during NMP								
Machine	Bio-Console 560	Prototype OrganOx	Bio-Console 560	Kidney Assist (Organ Assist)	Cardiopulmonary bypass technology	Kidney Assist (Organ Assist)	Kidney Assist (Organ Assist)	Kidney Assist (Organ Assist)
Pump	Centrifugal	Centrifugal	Centrifugal	Centrifugal	Roller	Centrifugal	Centrifugal	Centrifugal
Temperature	37 °C	37 °C	37 °C	37 °C	37 °C	From 8 °C to 35 °C (within 90 min)	37 °C	37 °C
Pressure	Flow controlled up to 75 mm Hg	Flow controlled, between 70–100 mm Hg	70–80 mm Hg	70 mm Hg	75–85 mm Hg	From 30 to 75 mm Hg (within 90 min)	Increased from 60 mm Hg, if necessary up to 100 mm Hg	Pressure controlled at 75 mm Hg
Oxygenation	95% O_2/5% CO_2 at 0.1 L/min	Proportional control valves; 10 ml/min; pO_2 10–26 kPa and pCO_2 2–6 kPa	95% O_2/5% CO_2	95% O_2/5% CO_2	95% O_2/5% CO_2 at 1.5 L/min	pO_2 values >500 mm Hg	100% O_2	95% O_2/5% CO_2 at 0.5 L/min
System	Closed	Closed	Closed	Open	Open	Open	Open	Open
Duration of NMP, h	1	24	3	6	1–3	1.5	2	6
Baseline perfusate additives before NMP start (per liter)								
Total perfusate volume, L	~0.6–0.7	0.5	0.5	2	0.75	2	0.75–1.25	1
Oxygen carrier	RBC 430–500 ml/L	RBC 500 ml/L	RBC 500 ml/L	RBC or HBOC 250 ml/L	RBC 334 ml/L	STEEN solution 250 ml/L	RBC 188–313 ml/L	RBC 500 ml/L
Isotonic solution	Ringer's solution 500–570 ml/L	None	Plasma-Lyte A 500 ml/L	Williams E Media 750 ml/L	Ringer's solution 200 ml/L Sterile water 33.4 ml/L	Ringer's solution 250 ml/L	Sterofundin solution 667–1250 ml/L	Sodium chloride 500 ml/L
Colloid	—	HSA 500 ml of 5%/L	—	—	Gelofusine 333 ml of 40%/L	—	—	HSA 100 ml of 20%/L
Other additives	Sodium bicarbonate 8.4% titrated to pH of 7.3–7.4	Sodium bicarbonate 8.4% 10.0–30.0 mmol/L	Sodium bicarbonate 8.4% 52.4 mmol/L	Sodium bicarbonate 8.4% titrated to pH of 7.3–7.4	Sodium bicarbonate 8.4% 20.0 mmol/L	Sodium bicarbonate 8.4% 11.0 mmol/L	Sodium bicarbonate 8.4% 10.3–420.0 mmol/L	Sodium bicarbonate 8.4% 20.0–30.0 mmol/L
	—	Calcium gluconate 10% 4.4 mmol/L	—	Calcium gluconate 10% titrated to 1.1–1.4 mmol/L	Calcium gluconate 10% 1.5 mmol/L	Calcium gluconate 10% 0.8 mmol/L	—	Calcium gluconate 10% 4.4 mmol/L
	Mannitol 10% 19.9–23.2 mmol/L	Mannitol 10% 11.1 mmol/L	—	—	Mannitol 10% 36.7 mmol/L	—	Mannitol 10% 4.17–13.9 mmol/L	Mannitol 10% 11.1 mmol/L
	—	Cefuroxime 1.5 g/L	—	—	—	Ampicillin 0.5 g/L	Augementin 9–15 g/L	Cefazolin 2 g/L
	Heparin 2865–3334 IU/L	—	Heparin 4000 IU/L	Heparin 1000 IU/L	Heparin 2670 IU/L	—	Heparin 1500–5000 IU/L	—
	Dexamethasone 11.4–13.3 mg/L	—	—	Dexamethasone 8 mg/L	—	—	Dexamethasone 6–8 mg/L	—

(*Continued on following page*)

Table 1 | (Continued) **Overview of the hardware (perfusion control mode, pressure, and gas supply), the perfusate composition, and supplements provided during NMP of the kidney for the different protocols**[2–5,10–13]

Perfusate compositions	Hosgood *et al.* (2013, 2017)	Weissenbacher *et al.* (2018)	Kabagambe *et al.* (2018)	Aburawi *et al.* (2019)	Hameed *et al.* (2019)	Minor *et al.* (2020)	Rijkse *et al.* (2021)	Arykbaeva *et al.* (NCT04693325)
Continuous perfusate additives during NMP (calculated per hour)								
Metabolic substrate								
Amino acids (total)	Synthamin 17 1.89 g/h with 3.44 mmol/L sodium bicarbonate	Nutriflex (only amino acids) 0.14 g[a]	Clinimex E 2.75/ 10 0.55 g/h	Williams E Media 0.305 g/h	M199 0.014 g/h	—	Nutriflex 1.15 g/h with 3.44 mmol/L sodium bicarbonate	Aminoplasmal 10% 2.33 g/h
of which glutamine	0 g/h	0.0123 g[a]	None	0.019 g/h	0.0013 g/h	—	0.01 g/h	1.68 g/h
Glucose	1.39–1.94 mmol/h	0.003 mmol[a]	11.1 mmol/h (dextrose)	Titrated to 5.5–11.1 mmol/L (dextrose) 4.16 mmol/h (in Williams E Media)	1.39 mmol/h (dextrose 5%) 0.11 mmol/h (in M199)	—	1.94 mmol/h	2.22 mmol/h
Insulin, IU/h	3.78	—	2	5	—	—	3.78	—
Multivitamins	Cernevit	—	Infutive Adult	(in Williams E Media)	(in M199)	—	Multivitamins 5 ml	Cernevit
Urine replacement	Ringer's solution	Recirculation	Plasma-Lyte A	Ringer's lactate	Ringer's solution	—	Recirculation	Recirculation
Vasodilator	Prostacyclin 0.05 mg/h	Prostacyclin 0.004 mg/h	—	—	Verapamil 12.5 mg/h	—	Prostacyclin 0.05 mg/h	Prostacyclin 0.009 mg/h

HBOC, hemoglobulin-based oxygen carrier; NMP, normothermic machine perfusion; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; RBC, red blood cell.
With the exception, successful transplantations in the studies of Hosgood *et al.*[2,9] (following 1-hour NMP), Minor *et al.*[10] (following 2 hours continuous rewarming), and Rijkse *et al.*[3] (following 2 hours NMP), none of the perfused kidney grafts have been transplanted. See Supplementary Table S1 for a specification of solutions used.
[a]When glucose <4 mmol/L.

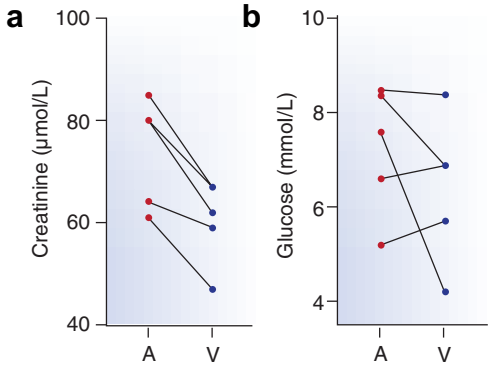

Figure 1 | Arteriovenous measurements of glucose and creatinine concentrations in prerenal (arterial [*A*]; red) and postrenal (venous [*V*]; blue) blood samples of (healthy) living kidney donors (n = 5) in samples collected as described by Lindeman *et al.*,[16] measured in a standard manner by the Clinical Chemistry Laboratory. (**a**) The lower venous creatinine levels illustrate renal creatinine clearance. (**b**) Varying glucose uptake among the different donors reflects a heterogeneous carbohydrate turnover. Adapted from 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*. 2020;98:1476–1488.[16]

insulin-independent. An exception is the insulin-dependent GLUT4, which controls postprandial glucose disposal.[37] Because GLUT4 is also expressed in the kidney,[16,38] insulin supplementation during NMP should be considered.

Apart from the lactate uptake, the arteriovenous concentration differences show that the kidney also clears organic acids, such as citrate and malate, from the circulation.[39] Both are direct intermediates in the citric acid cycle. For citrate, it has been speculated that this clearance reflects a urinary excretion mechanism as part of a citrate disposal system.[40] Yet, tracer studies in mice[15] and persistent citrate clearance by transiently anuric deceased donor grafts in the phase immediately following transplantation imply that citrate is, at least partially, metabolized by the kidney rather than just secreted in the urine.[16] Consequently, citrate could be considered a carbon source in the context of renal NMP.

Above observations imply a central role for the kidney in the physiological control of carbohydrate and organic acid homeostasis. In fact, clinical studies show that acute kidney injury associates with profound derangements in carbohydrate metabolism[15] that include a profoundly impaired lactate clearance. Along similar lines, differences in postreperfusion lactate dynamics (arteriovenous concentration differences) discriminate between the different levels of postreperfusion metabolic competence following kidney transplantation[16] (Figure 3[16]). Accordingly, one could speculate that lactate metabolism can be used as a readout of graft metabolic competence. In this respect, a progressive perfusate lactate accumulation observed in NMP experiments using human discarded kidney grafts[4,5,12,13] challenges the reinstatement of a physiological metabolic homeostasis under the current NMP conditions.

Although the lactate accumulation during NMP could reflect disposal of red blood cell–produced lactate (either through direct lactate oxidation and/or alternatively lactate conversion to glucose [Cori cycle]),[33] it is likely that the lactate accumulation largely relates to ongoing renal lactate production as result of impaired oxidative phosphorylation under the current NMP conditions. Accruing perfusate lactate levels will result in metabolic acidosis. Although this acidosis can be efficiently corrected by titrating bicarbonate to the perfusate, lactate accumulation may result in product-inhibition of renal and erythrocyte lactate dehydrogenase activity, and as a consequence an impaired reduction of formed pyruvate to lactate. This final step in the glycolytic pathway is critical for maintaining oxidation-reduction neutrality by regenerating nicotinamide adenine dinucleotide positive (NAD^+) from the reduced NAD formed during glycolysis.[41] As a consequence, lactate dehydrogenase product inhibition resulting from the accruing lactate levels may result in cellular oxidation-reduction stress.

The kidney not only shares this gluconeogenetic capability with the liver, but it also has the capacity to store glycogen, albeit less equipped with a glycogenolytic system compared with that of the liver. As a consequence, the role of renal glycogenolysis in maintaining blood glucose levels during

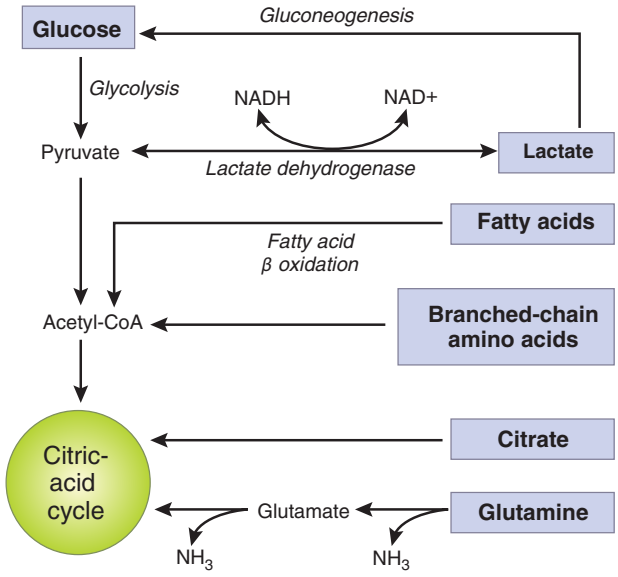

Figure 2 | Schematic overview of the metabolic substrates utilized by the kidney in their simplified pathways. Under normal conditions, lactate is used for gluconeogenesis, and glucose, fatty acids, glutamine (amino acids), and possibly citrate are used to fuel the oxidative phosphorylation. Under hypoxic conditions, or under conditions of high-energy demand, glycolysis is activated, resulting in a net release of lactate from the kidney. NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NH_3, ammonia. Partially adapted from Mather A, Pollock C. Glucose handling by the kidney. *Kidney Int*. 2011;79:(Suppl 120):S1–S6.[25]

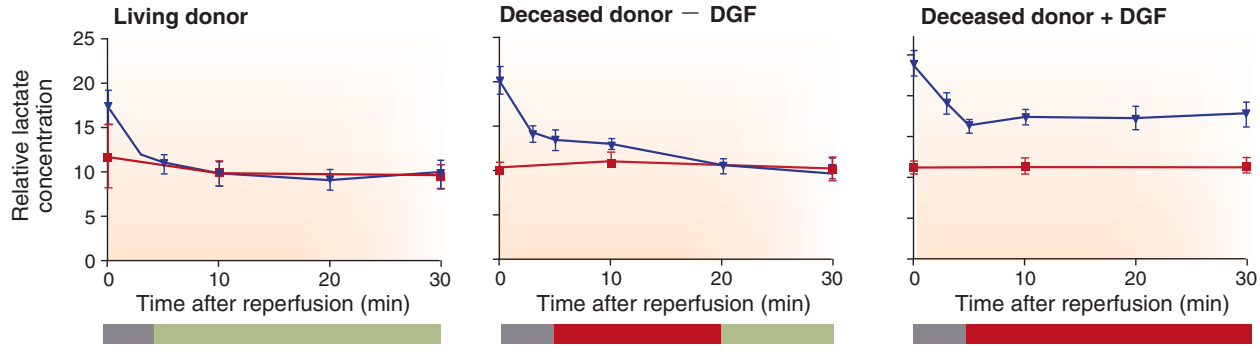

Figure 3 | Lactate dynamics illustrating the different levels of renal metabolic competence following kidney transplantation. Curves reflect the relative renal arterial (red) and venous (blue) lactate levels. Initial washout of lactate accumulated during storage (indicated by the gray bar) and immediate (living donor) or delayed (deceased donor graft without delayed graft function [DGF]) suppression of glycolysis (indicated by the green bar). Transient persistent normoxic glycolysis in deceased donor grafts without and with DGF (indicated by the red bar). Adapted from 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*. 2020;98:1476–1488.[16]

intermittent fasting is reported to be limited.[42,43] Still, because inverse associations are found between kidney glycogen stores and the extent of ischemic kidney injury, glycogen stores may serve as an endogenous energy buffer under extreme conditions, such as graft procurement and transplantation.[42,44] Consequently, it could be speculated that the preservation, or even reestablishment, of renal glycogen stores by NMP is beneficial.

Fatty acids

Although the current perfusion protocols are essentially glucose centered, *in vivo* studies show that the kidney, like the heart, relies on fatty acids as primary fuel source.[17] Yet, although the heart has a clear preference for long-chain (C16 and longer) fatty acids,[18] the kidney essentially fuels on medium-chain fatty acids (MCFAs) (Figure 4[16] and Supplementary Table S2).

The renal preference for MCFAs has several advantages over long-chain fatty acids, as they do not rely on specific transmembrane transporters to cross the plasma and mitochondrial membranes by specific transporters and carnitine shuttling.[45] So, MCFAs can simply diffuse through the inner mitochondrial membrane, where the oxidation of the fatty acids occurs.[46] With the aim of optimally mimicking renal metabolic physiology during NMP, provision of MCFAs during NMP should be considered. In this context, most parenteral lipid emulsion formulations are essentially long-chain fatty acid based and are therefore not optimally suited for renal NMP. However, MCFA-enriched formulas are available: Lipofundin (MCFA 10%) and Smoflipid (MCFA 6%). Given the apparent minimal oxidation of long-chain fatty acids by the kidney, further enrichment of MCFAs to these solutions might be preferable.

Lipid delivery during NMP comes with several challenges. The vast majority of plasma fatty acids are carried by albumin, with unbound fatty acids representing only a minor fraction (<0.01%) of the total.[45] More important, clinical-grade albumin, most often used in the NMP protocols, has not undergone any pretreatment to free fatty acid binding sites. As a result, binding sites will be occupied mainly by the naturally dominating long-chain fatty acids. Furthermore, NMP is performed in an isolated, closed, and limited volume setup. As a consequence, the system has a limited lipid buffering capacity (because no adipose tissue, muscle, and liver are included), which imposes challenges with respect to the maintenance of MCFA supply and potential lipotoxicity. In the light of the limited free fatty acid carrying capacity of plasma or its derivates, it could be argued that preference should be given to lauric acid (C12:0) enrichment, because this MCFA has the highest per molecule energy content.

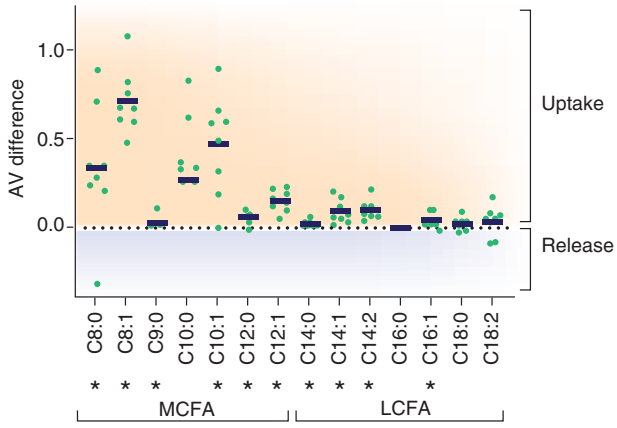

Figure 4 | Arteriovenous (AV) concentration differences in (healthy) living donor kidneys for middle-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs), illustrating the preference of the kidney for MCFAs.[16] *Significant uptake (paired *t* test). Adapted from Lindeman JH, Wijermars LG, Kostidis S, et al. Results of an explorative clinical evaluation suggest immediate and persistent post-reperfusion metabolic paralysis drives kidney ischemia reperfusion injury. *Kidney Int*. 2020;98:1476–1488.[16]

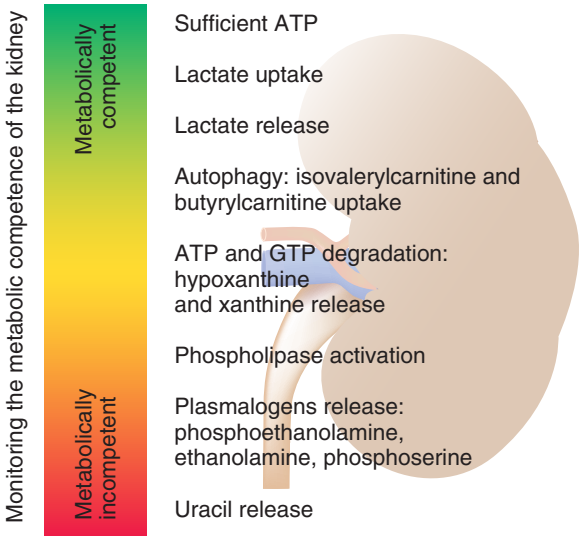

Figure 5 | Gauging metabolic status. Schematic overview illustrating a cluster of liquid biomarkers that can be applied to monitor metabolic status during renal normothermic machine perfusion. ATP, adenosine triphosphate; GTP, guanosine triphosphate.

Apart from their role as an energy source, provision of other lipid classes critical to cellular function, such as essential unsaturated fatty acids and cholesterol, should be considered during prolonged NMP, in particular in protocols that aim at organ regeneration. However, inclusion of unsaturated fatty acids in the NMP protocols carries specific risks. Unsaturated fatty acids may promote oxidative stress and lipid peroxidation, particularly in the presence of excess free hemoglobin/heme released as a consequence of hemolysis[47] during the perfusion or when using heme-based oxygen carriers.[48]

Amino acids

Although amino acids are generally viewed as building blocks for protein synthesis, oxidation of external (plasma) or endogenous (autophagy) amino acids is an integral part of the normal metabolic plasticity.[49–51] As a consequence, an adequate amino acid supply is a critical requisite for both the anabolic aspects (including the replacement of proteins lost as result of activated autophagy that occurred in the donation process) during NMP as well as catabolic substrates. With respect to the latter, in particular, glutamine and to a lesser extent the branched-chain amino acids constitute as bona fide catabolic substrates that are part of the normal metabolic plasticity[52] (Figure 2).

Glutamine is the most abundant plasma amino acid, and is a significant carbon source. In fact, glutamine contributes to up to 10% to 20% of the overall glucose production in the body.[53] Arteriovenous concentration differences show significant glutamine uptake by porcine kidneys, but no uptake was observed in human donor kidneys (immediately before cross clamping in the process of living-donor organ donation).[16,18] Although this may reflect a species differences, it may also reflect differences in the metabolic status during the pig experiments, and the conditions of live donation (preoperative starvation, anesthesia, and routine glucose infusions) under which the arteriovenous sampling in humans has been performed.[16,18] Note that significant renal glutamine (and branched-chain amino acid) oxidation occurs in the first minutes of graft reperfusion and is more prolonged during delayed graft function (DGF). This suggests that glutamine oxidation does occur in humans under more extreme conditions, and that metabolic adaptations occur within minutes.

Considering its established role in metabolic homeostasis and plasticity, glutamine supplementation should be considered during NMP. Note that for stability reasons, parenteral nutrition compositions contain glutamic acid, the precursor for glutamine, rather than glutamine (Table 1). The conversion of glutamate to glutamine requires glutamine synthetase, which is expressed in the kidney.[54] However, the conversion relies on an adequate adenosine triphosphate (ATP) supply and may thus be impaired under metabolically compromised conditions. Therefore, direct glutamine supplementation should be maintained and might be preferable for NMP.

Amino acid oxidation comes at the expense of ammonia (NH_3) production. Ammonia produced in the kidney is normally either excreted directly into the urine with a parallel (equimolar) formation of bicarbonate[53] or converted to urea in the liver.[55,56] Yet, ammonia may accumulate during prolonged NMP. Although ammonia-mediated neural toxicity[57] is presumably not an issue during NMP, accumulated ammonia may impair the acid-base balance, and consequently excess ammonia accumulation should be avoided. Hence, longer perfusions, in particular of protocols with urine recirculation,[5] may require some form of ammonia disposal (e.g., dialysis).

Micronutrients

Micronutrients (vitamins and minerals) are crucial for maintaining tissue homeostasis. The importance of an adequate provision of B vitamins for metabolic homeostasis is illustrated by findings that thiamine (vitamin B1) supplementation preserves renal carbohydrate metabolism during acute kidney injury by enhancing glucose production and decreasing the glycolysis rate.[15] Furthermore, it is anticipated that oxidation-reduction stress with a shift in the NAD/reduced NAD balance occurs during ischemia and ischemia-reperfusion injury. Nicotinamide riboside (vitamin B3) and nicotinamide mononucleotide–NAD^+ precursors have been shown to effectively replete compromised NAD^+ levels in general[58–60] as well as during ischemia.[61] Based on these observations, thiamine and nicotinamide riboside or nicotinamide mononucleotide supply should be included in NMP protocols aimed at prolonged perfusions.

Other vitamins to be considered during prolonged perfusions include antioxidants, such as vitamins C and E, as well as cell differentiation factors, such as vitamins A and D.[62,63] Inclusion of vitamins C and E could improve antioxidant status. However, they can also have toxic effects in the

presence of free iron or heme/hemoglobin, and therefore their inclusion in NMP protocols requires careful consideration.[47]

A further aspect that merits attention is the repletion of the purine pool. Organ procurement, even in living donor donation procedures, is accompanied by adenosine (ATP) and guanosine triphosphate (GTP) depletion, as reflected by the washout of their breakdown product (hypo)xanthine in the first minutes of reperfusion, following transplantation.[16] This depletion may compromise the ATP/GTP pool, critically impeding metabolic homeostasis. Although both are nonessential molecules, their *de novo* synthesis comes with an extreme energy burden (6 ATPs per molecule), making their supplementation essential. A dual approach has been proposed, combining inhibition of adenosine/guanine oxidation through the xanthine oxidase inhibitor allopurinol with inosine supplementation, a direct precursor of adenosine/guanine.[64] Of note, direct adenosine supplementation is not feasible because of its comprehensive autocrine, paracrine, and hormonal effects, and its rapid catabolism by extracellular adenosine deaminases.

Finally, although most current NMP protocols include calcium[65] and magnesium,[66] supplementation of other trace metals should be considered during more prolonged perfusion periods. For example, iron[67] is an indispensable element of cytochromes and catalase, whereas selenium constitutes the central factor of the antioxidant enzyme glutathione peroxidase.[68] Plasma could be considered as a potential substrate for the perfusate as a good source for trace elements and free iron/heme reducing elements, which is already implemented in liver NMP.[69]

Monitoring metabolic competence

Optimization of NMP and graft viability testing critically relies on monitoring of the metabolic status as functional readout. Based on the observations from the postreperfusion metabolomic profiles of kidneys with and without DGF,[16] some practical recommendations can be made with respect to readouts for monitoring the metabolic status during kidney NMP.

The most straightforward way to assess metabolic homeostasis is to directly monitor the graft's ATP content. ATP probes for monitoring tissue ATP content are commercially available, yet these probes rely on an enzyme-coated surface, making them incompatible with clinical applications.[70] Because of the extreme short half-life of ATP, any measurement in tissue biopsies will rely on snap frozen samples, a strategy that is not compatible with clinical transplantation. Although tissue phosphocreatine can be used as a more stable surrogate for assessing graft ATP levels and does not require clamp freezing,[16] monitoring will rely on sequential collection of tissue biopsies. Alternatively, 31P magnetic resonance spectroscopy/magnetic resonance imaging can be applied to measure high-energy phosphate status,[71] yet this technique requires an NMP setup that is fully compatible with the conditions of magnetic resonance imaging.

Less invasive, but more indirect, readouts of metabolic competence include perfusate uracil accumulation as measure of tissue damage,[72] (hypo)xanthine release as measure of purine (ATP and GTP) catabolism,[73] and release of plasmalogens reflecting renal phospholipase C/D activation as a consequence of ATP exhaustion.[74] More subtle liquid biomarkers, reflecting a suboptimal metabolic state, include lactate *release* (ongoing glycolysis), isovalerylcarnitine accumulation as marker of activated autophagia,[51] and lactate *uptake* (reflecting metabolic competence).[15] The metabolic continuum of these liquid biomarkers for monitoring metabolic competence is summarized in Figure 5. More detailed monitoring of the more subtle/discrete (sub)homeostatic status and metabolic fluxes will rely on more sophisticated tools, such as isotope labeling.[75]

Conclusion

NMP is a promising development in the context of kidney graft quality assessment as well as *ex situ* viability enhancement. Although NMP has been proven feasible and safe in clinical studies, it could be argued that the currently applied perfusion protocols are not optimized in addressing the specific needs of the kidney in the absence of the body's integrated homeostatic system, which provides maximal metabolic plasticity (substrate delivery and/or disposal). This review provides a theoretical framework for more kidney-tailored perfusion protocols. Although glucose is generously administered in all reported protocols, it could be argued that more physiological concentrations are preferable. The need for concomitant insulin supplementation requires evaluation. Accruing lactate levels observed under the current NMP protocols imply the need for inclusion of auxiliary dialysis device in the NMP setup to avoid cellular oxidation-reduction stress. Amino acid mixtures included in some perfusion protocols are not tailored to the specific needs of renal NMP. In the context of achieving a more optimal metabolic plasticity, glutamine supplementation should be considered. Physiological studies show that MCFAs may constitute a primary fuel source for the kidney. However, their inclusion in perfusion protocols comes with specific challenges and potential toxicity concerns. Consequently, the potential advantages of MCFA enrichment (physiologically preferred substrate, high energy density, and lower lactate burden) should be balanced against the need for developing a specific delivery platform.

It is unlikely that metabolic recovery per se can be used a discriminatory tool for graft viability in short perfusion protocols. Clinical DGF is preceded by a metabolic collapse with activated normoxic glycolysis and persistent high-energy nucleotide catabolism following reperfusion.[16] However, because registry data show that DGF (in donation after cardiac death grafts) does not impact graft survival,[76] sole reliance on (reinstatement) of metabolic competence as a readout of graft viability may result in unjustified decisions to (not) transplant an organ.

Although NMP has great potential and the pioneering studies show that NMP is technically feasible and clinically compatible, establishment of the most optimal NMP protocol is a critical prerequisite for NMP to meet the 4Rs. Achieving metabolic competent state and recruitment of physiological potential during NMP may address part of the problems encountered in the current protocols (lactate accumulation and ammonium and heme toxicity). It is anticipated that the optimizing process will require systematic evaluation of multiple aspects, an endeavor that will rely on appropriate metabolic readouts of kidney damage, alignment of perfusion protocols to allow cross-study comparisons, and thorough validation studies with appropriate clinical end points (i.e., organ utilization, incident primary nonfunction, and graft survival; and for donation after brain death [DBD] [but not for donation after cardiac death donors[76]] incident DGF).

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Description of solution components.

Table S2. Arteriovenous difference of free fatty acids.

REFERENCES

1. De Meester J. The expanded criteria donor for kidney transplant: not a nearly new car. *Transplant Int*. 2017;30:11–13.
2. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013;13:1246–1252.
3. Rijkse E, de Jonge J, Kimenai HJAN, et al. Safety and feasibility of 2 h of normothermic machine perfusion of donor kidneys in the Eurotransplant Senior Program. *BJS Open*. 2021;5:zraa024.
4. Hameed AM, Lu DB, Patrick E, et al. Brief normothermic machine perfusion rejuvenates discarded human kidneys. *Transplant Direct*. 2019;5:e502.
5. Weissenbacher A, Lo Faro L, Boubriak O, et al. Twenty-four-hour normothermic perfusion of discarded human kidneys with urine recirculation. *Am J Transplant*. 2019;19:178–192.
6. Tingle SJ, Figueiredo RS, Moir JAG, et al. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. *Cochrane Database Syst Rev*. 2019;3:CD011671.
7. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med*. 2009;360:7–19.
8. Rijkse E, Ijzermans JN, Minnee RC. Machine perfusion in abdominal organ transplantation: current use in the Netherlands. *World J Transplant*. 2020;10:15–28.
9. Hosgood SA, Nicholson ML. First in man renal transplantation after ex vivo normothermic perfusion. *Transplantation*. 2011;92:735–738.
10. Minor T, von Horn C, Gallinat A, et al. First-in-man controlled rewarming and normothermic perfusion with cell-free solution of a kidney prior to transplantation. *Am J Transplant*. 2020;20:1192–1195.
11. Hosgood SA, Saeb-Parsy K, Wilson C, et al. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. *BMJ Open*. 2017;7:e012237.
12. Aburawi MM, Fontan FM, Karimian N, et al. Synthetic hemoglobin-based oxygen carriers are an acceptable alternative for packed red blood cells in normothermic kidney perfusion. *Am J Transplant*. 2019;19:2814–2824.
13. Kabagambe SK, Palma IP, Smolin Y, et al. Combined ex vivo hypothermic and normothermic perfusion for assessment of high-risk deceased donor human kidneys for transplantation. *Transplantation*. 2019;103:392–400.
14. Elliott TR, Nicholson ML, Hosgood SA. Normothermic kidney perfusion: an overview of protocols and strategies. *Am J Transplant*. 2021;21:1382–1390.
15. 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;2:732–743.
16. 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*. 2020;98:1476–1488.
17. Wang Z, Ying Z, Bosy-Westphal A, et al. Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure. *Am J Clin Nutr*. 2010;92:1369–1377.
18. Jang C, Hui S, Zeng X, et al. Metabolite exchange between mammalian organs quantified in pigs. *Cell Metab*. 2019;30:593–606.e3.
19. Yudkin J, Cohen R. The contribution of the kidney to the removal of a lactic acid load under normal and acidotic conditions in the conscious rat. *Clin Sci*. 1975;48:121–131.
20. Scheffer M, Bolhuis JE, Borsboom D, et al. Quantifying resilience of humans and other animals. *Proc Natl Acad Sci U S A*. 2018;115:11883–11890.
21. Lopez-Otin C, Kroemer G. Hallmarks of health. *Cell*. 2021;184:33–63.
22. Glatz JFC, Nabben M, Young ME, et al. Re-balancing cellular energy substrate metabolism to mend the failing heart. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165579.
23. Banke NH, Wende AR, Leone TC, et al. Preferential oxidation of triacylglyceride-derived fatty acids in heart is augmented by the nuclear receptor PPARalpha. *Circ Res*. 2010;107:233–241.
24. Alsahli M, Gerich JE. Renal glucose metabolism in normal physiological conditions and in diabetes. *Diabetes Res Clin Pract*. 2017;133:1–9.
25. Mather A, Pollock C. Glucose handling by the kidney. *Kidney Int*. 2011;79(Suppl 120):S1–S6.
26. Uchida S, Endou H. Substrate specificity to maintain cellular ATP along the mouse nephron. *Am J Physiol*. 1988;255(pt 2):F977–F983.
27. Stumvoll M, Chintalapudi U, Perriello G, et al. Uptake and release of glucose by the human kidney: postabsorptive rates and responses to epinephrine. *J Clin Invest*. 1995;96:2528–2533.
28. Stumvoll M, Meyer C, Mitrakou A, et al. Renal glucose production and utilization: new aspects in humans. *Diabetologia*. 1997;40:749–757.
29. Epstein FH. Oxygen and renal metabolism. *Kidney Int*. 1997;51:381–385.
30. Bagnasco S, Good D, Balaban R, et al. Lactate production in isolated segments of the rat nephron. *Am J Physiol*. 1985;248(pt 2):F522–F526.
31. Guder WG, Ross BD. Enzyme distribution along the nephron. *Kidney Int*. 1984;26:101–111.
32. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol*. 2004;558(pt 1):5–30.
33. Rodwell V, Bender D, Botham K, et al. *Harper's Illustrated Biochemistry*. 31st ed. New York, NY: McGraw-Hill Education LCC; 2018.
34. Wirthensohn G, Guder WG. Renal substrate metabolism. *Physiol Rev*. 1986;66:469–497.
35. de Boer IH, Kahn SE. SGLT2 inhibitors-sweet success for diabetic kidney disease? *J Am Soc Nephrol*. 2017;28:7–10.
36. Gronda E, Jessup M, Iacoviello M, et al. Glucose metabolism in the kidney: neurohormonal activation and heart failure development. *J Am Heart Assoc*. 2020;9:e018889.
37. Klip A, McGraw TE, James DE. Thirty sweet years of GLUT4. *J Biol Chem*. 2019;294:11369–11381.
38. Vallon V. Glucose transporters in the kidney in health and disease. *Pflugers Arch*. 2020;472:1345–1370.
39. Hamm LL. Renal handling of citrate. *Kidney Int*. 1990;38:728–735.
40. Simpson DP. Citrate excretion: a window on renal metabolism. *Am J Physiol*. 1983;244:F223–F234.
41. Nielsen VK, Kemp E, Laursen T. Lactic dehydrogenase in kidney tissue and renal disease: adaptive change of the synthesis in acute failure. *Acta Med Scand*. 1968;184:109–119.
42. Haase VH. Got glycogen? an energy resource in HIF-mediated prevention of ischemic kidney injury. *Kidney Int*. 2020;97:645–647.
43. Jongbloed F, Saat TC, Verweij M, et al. A signature of renal stress resistance induced by short-term dietary restriction, fasting, and protein restriction. *Sci Rep*. 2017;7:40901.

44. Ito M, Tanaka T, Ishii T, et al. Prolyl hydroxylase inhibition protects the kidneys from ischemia via upregulation of glycogen storage. *Kidney Int*. 2020;97:687–701.
45. Schonfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. *J Lipid Res*. 2016;57:943–954.
46. Kompare M, Rizzo WB. Mitochondrial fatty-acid oxidation disorders. *Semin Pediatr Neurol*. 2008;15:140–149.
47. Berger HM, Lindeman JHN, van Zoeren-Grobben D, et al. Iron overload, free radical damage, and rhesus haemolytic disease. *Lancet*. 1990;335: 933–936.
48. Chiabrando D, Vinchi F, Fiorito V, et al. Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. *Front Pharmacol*. 2014;5:61.
49. Garibotto G, Sofia A, Saffioti S, et al. Amino acid and protein metabolism in the human kidney and in patients with chronic kidney disease. *Clin Nutr*. 2010;29:424–433.
50. Tang C, Livingston MJ, Liu Z, et al. Autophagy in kidney homeostasis and disease. *Nat Rev Nephrol*. 2020;16:489–508.
51. Beese CJ, Brynjolfsdottir SH, Frankel LB. Selective autophagy of the protein homeostasis machinery: ribophagy, proteaphagy and ER-phagy. *Front Cell Dev Biol*. 2019;7:373.
52. Young GA. Amino acids and the kidney. *Amino Acids*. 1991;1:183–192.
53. Stumvoll M, Perriello G, Meyer C, et al. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. *Kidney Int*. 1999;55:778–792.
54. Taylor L, Curthoys NP. Glutamine metabolism: role in acid-base balance*. *Biochem Mol Biol Educ*. 2004;32:291–304.
55. Weiner ID, Verlander JW. Renal ammonia metabolism and transport. *Compr Physiol*. 2013;3:201–220.
56. Machado MC, Pinheiro da Silva F. Hyperammonemia due to urea cycle disorders: a potentially fatal condition in the intensive care setting. *J Intensive Care*. 2014;2:22.
57. Barrett KE, Chapter 14: ammonia and urea. *Gastrointestinal Physiology*. 2nd ed. New York, NY: The McGraw-Hill Companies; 2014. Available at: https://accessmedicine.mhmedical.com/content.aspx?bookid=691&sectionid=45431415. Accessed April 30, 2021.
58. Trammell SA, Schmidt MS, Weidemann BJ, et al. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun*. 2016;7:12948.
59. Schondorf DC, Ivanyuk D, Baden P, et al. The NAD+ precursor nicotinamide riboside rescues mitochondrial defects and neuronal loss in iPSC and fly models of Parkinson's disease. *Cell Rep*. 2018;23:2976–2988.
60. Rajman L, Chwalek K, Sinclair DA. Therapeutic potential of NAD-boosting molecules: the in vivo evidence. *Cell Metab*. 2018;27:529–547.
61. Yamamoto T, Byun J, Zhai P, et al. Nicotinamide mononucleotide, an intermediate of NAD+ synthesis, protects the heart from ischemia and reperfusion. *PLoS One*. 2014;9:e98972.
62. Mihajlovic M, Fedecostante M, Oost MJ, et al. Role of vitamin D in maintaining renal epithelial barrier function in uremic conditions. *Int J Mol Sci*. 2017;18:2531.
63. Mallipattu SK, He JC. The beneficial role of retinoids in glomerular disease. *Front Med (Lausanne)*. 2015;2:16.
64. Szoleczky P, Modis K, Nagy N, et al. Identification of agents that reduce renal hypoxia-reoxygenation injury using cell-based screening: purine nucleosides are alternative energy sources in LLC-PK1 cells during hypoxia. *Arch Biochem Biophys*. 2012;517: 53–70.
65. Han HJ, Park SH, Lee YJ, et al. Effect of ATP on Ca2+ uptake in the presence of high glucose in renal proximal tubule cells. *Clin Exp Pharmacol Physiol*. 2003;30:694–701.
66. Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J*. 2012;5(suppl 1):i3–i14.
67. Swaminathan S. Iron homeostasis pathways as therapeutic targets in acute kidney injury. *Nephron*. 2018;140:156–159.
68. Iglesias P, Selgas R, Romero S, et al. Selenium and kidney disease. *J Nephrol*. 2013;26:266–272.
69. Matton APM, Burlage LC, van Rijn R, et al. Normothermic machine perfusion of donor livers without the need for human blood products. *Liver Transpl*. 2018;24:528–538.
70. Llaudet E, Hatz S, Droniou M, et al. Microelectrode biosensor for real-time measurement of ATP in biological tissue. *Anal Chem*. 2005;10: 3267–3273.
71. Liu Y, Gu Y, Yu X. Assessing tissue metabolism by phosphorous-31 magnetic resonance spectroscopy and imaging: a methodology review. *Quant Imaging Med Surg*. 2017;7:707–716.
72. Johnson TA, Jinnah HA, Kamatani N. Shortage of cellular ATP as a cause of diseases and strategies to enhance ATP. *Front Pharmacol*. 2019;10:98.
73. Lecca D, Ceruti S. Uracil nucleotides: from metabolic intermediates to neuroprotection and neuroinflammation. *Biochem Pharmacol*. 2008;75: 1869–1881.
74. Boutilier RG. Mechanisms of cell survival in hypoxia and hypothermia. *J Exp Biol*. 2001;204:3171–3181.
75. Jang C, Chen L, Rabinowitz JD. Metabolomics and isotope tracing. *Cell*. 2018;173:822–837.
76. de Kok MJ, McGuinness D, Shiels PG, et al. The neglectable impact of delayed graft function on long-term graft survival in kidneys donated after circulatory death associates with superior organ resilience. *Ann Surg*. 2019;270:877–883.