International Journal of Surgery Open 23 (2020) 39–47

Contents lists available at ScienceDirect

International Journal of Surgery Open

journal homepage: www.elsevier.com/locate/ijso

Research Paper

Cold ischaemia time: Is too long really too bad? Studies using a porcine kidney ex-vivo reperfusion model

Karim Hamaoui [a, *], Sally Gowers [b], Bynvant Sandhu [a], Terry Cook [c], Martyn Boutelle [b], Daniel Casanova-Rituerto [a, d], Vassilios Papalois [a, c]

[a] Department of Surgery, Imperial College London, UK
[b] Department of Bioengineering, Imperial College London, UK
[c] Imperial College Renal and Transplant Centre, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK
[d] Department of Surgery, University of Cantabria, Spain

ARTICLE INFO

Article history:
Received 21 August 2019
Received in revised form
29 September 2019
Accepted 15 October 2019
Available online 24 October 2019

Keywords:
Cold ischaemia time
Preservation
Machine perfusion
Extended criteria
Hypothermia
Viability assessment

ABSTRACT

Introduction: Post-ischaemic hypothermic machine perfusion (HMP) may be beneficial in recovery of marginal kidney grafts. The full capacity of conventional HMP (with passive oxygenation) to recondition an organ has not been realised. We investigated whether HMP can ameliorate ischemic damage caused by extremely prolonged static cold storage (SCS).

Methods: Porcine kidneys underwent 4-h (SCS4,n = 4) or 52-h (SCS52,n = 4) SCS, followed by 10 h of HMP and were then subjected to 2 h of isolated normothermic reperfusion (NRP).

Results: There was a post-SCS graft weight loss in SCS52 vs SCS4 kidneys. SCS52 kidneys showed viable perfusion dynamics during HMP, with significantly shorter times to reach viable parameters vs SCS4 kidneys ($p < 0.027$). During NRP SCS52 kidneys demonstrated similar trends in perfusion dynamics, renal function, oxygen consumptions, lactate production, and tubular injury to SCS4 kidneys.

Conclusion: Graft weight loss after SCS, reducing resistance to perfusion, may facilitate better HMP dynamics and graft reconditioning. Clinicians utilising HMP should be aware of this phenomenon when using HMP in kidneys exposed to extreme periods of SCS. HMP after an extended period of SCS can resuscitate kidneys to a level equitable of viability as those after a short period of SCS. Utilising passive oxygenation however may be limiting such recovery and interventions utilising active oxygenation may provide benefit in such organs.

1. Introduction

Despite the growing evidence for benefits of hypothermic machine perfusion (HMP) in allograft preservation implementation of HMP protocols similar to those described in the Eurotransplant Trial is logistically complex and resource intensive [1]. The necessity of this technology being timely available at the site of organ retrieval for transport to the recipient centre, as well as continuous monitoring during preservation and transport, has limited uptake of HMP for routine organ preservation, where static cold storage (SCS) may actually be sufficient for the majority of recovered organs [2]. Use of HMP likely lies in preservation of extended criteria donor (ECD) and donation after cardiac death (DCD) allografts for reconditioning [3] and assessing viability. Interest has focussed on post-ischaemic hypothermic reconditioning as a technique that may allow benefits of HMP to be realised in allografts after a period of SCS

Koetting et al. [4] first demonstrated the benefit of hypothermic reconditioning by HMP to recover organs after prolonged (18 h) SCS ischemic injury. HMP produced substantial 3-fold higher clearances of creatinine and urea, and less apoptotic caspase-3 activity during organ viability assessment on an isolated reperfusion circuit and effectively ameliorated energetic breakdown and deterioration of mitochondrial redox balance. Another study using a porcine renal autotransplantation model demonstrated that a short 2 h period of HMP reconditioning after prolonged (21 h) SCS proved as effective as continuous HMP for the whole storage time [5].

Clinical evidence further points to HMP as superior to SCS preservation ([6]). The development of HMP is being directed to

* Corresponding author.
E-mail address: karim.hamaoui08@imperial.ac.uk (K. Hamaoui).

https://doi.org/10.1016/j.ijso.2019.10.002

towards reconditioning and optimising viability of less viable DCD and ECD donor allografts with the intent to promote their immediate function, and increase longer term survival, allowing an expansion of the donor pool with these donor cohorts. Recently retrospective analysis of post ischaemic HMP vs CS alone in ECD grafts in the Eurotransplant region suggested improved rates of PNF, with HMP an independent factor in the prevention of DGF.

At present the full capacity of HMP to recondition an organ is unknown [5], but research investigating the limits to which HMP can be effectively used will allow a better application of HMP in clinical practice.

The objective of this study was to evaluate the effect of extreme cold ischaemia time (CIT) on perfusion parameters measured during HMP establish whether a period of post-ischaemic HMP is able to reverse the effects of prolonged CIT and restore renal function.

2. Methods

2.1. Experimental protocol

Eight porcine DCD (Maastricht Type-2 equivalent) kidneys retrieved from 4 land race abattoir pigs were used in this study [10]. One kidney of each pair underwent SCS for either 4 h (SCS4,n = 4) or 52 h (SCS52,n = 4), paired kidneys were compared to eliminate the impact of donor variability. All kidneys after SCS underwent 10 h of HMP reconditioning on a Waters Medical Systems RM3 device with UW solution. Heterogeneous clinical HMP pump times have been reported in the literature with averages varying between 10 [7] to 31 [8] hours. Thus 10 h of HMP was considered to be a reasonable starting point for reconditioning in this extreme CIT model to allow for stabilization of HMP parameters for viability interpretation. After HMP kidneys underwent viability assessment for 2 h using an oxygenated NRP circuit with KHB solution. Experiments were conducted in the laboratories of the Department of Surgery of Imperial College London.

2.2. Hypothermic machine perfusion

Kidneys were perfused hypothermically (5–7 °C) on two commercially available Waters Medical RM3 perfusion units (Rochester, MN, USA) for 10 h with 1L of UW Belzer MPS. Perfusion was based on clinical protocols [9] with a target systolic perfusion pressure of 40 mmHg by 15 min of perfusion.

2.3. Reperfusion circuit

After 10 h of HMP kidneys were connected to a NRP circuit to assess organ viability and has been previously described in detail [10].

2.4. Analytical methods

Real-time perfusion dynamics were measured and a perfusion flow index (PFI, ml/min/100 g/mmHg) was calculated for each kidney. During reperfusion creatinine clearance was calculated to determine glomerular filtration rate (GFR) per gram of tissue as (urinary creatinine × urinary volume/reperfusate creatinine). Urinary lactate dehydrogenase (LDH)/GFR ratio per gram of tissue was similarly calculated. Oxygen consumption was calculated as the (difference between arterial and venous partial pressure of oxygen) × flow rate/100 g of tissue.

2.5. Rapid sampling microdialysis

A rapid sampling microdialysis (rsMD) system was used to measure markers of ischaemia and metabolism during HMP and normothermic reperfusion, this was an in house system and has recently been described in detail [11]. Microdialysis probes (MAB11.35.4, Microbiotech, Stockholm, Sweden) were inserted superficially into the parenchyma of the lateral cortex at the start of HMP and connected to the microdialysis analyser. Tissue lactate levels were analysed in real-time to reveal information on interstitial lactate concentration.

2.6. Histological analysis

Needle core biopsies were taken pre-HMP, post-HMP, and wedge biopsies after 2 h of normothermic reperfusion, and stored in formalin. 4 μm cut sections were stained with H&E, (periodic acid-Schiff). Tubular damage was scored for: tubular dilation, tubular debris, and nuclear fragmentation of tubular cells; were scored over three fields using a semi-quantitative scale: 0(normal); 1(0–25% affected); 2(25–50% affected); 3(50%–75% affected) and 4 (>75% affected).

2.7. Statistical methods

Values are represented as mean and standard deviation. Continuous variables from paired contra-lateral kidneys were evaluated using the paired students T-test or non-parametric Wilcoxon signed rank test. Data and statistical analysis was performed using Microsoft® Excel Software (Reading, UK). All tests were 2-tailed and a p value of ≤ 0.05 was considered significant.

3. Results

3.1. Hypothermic machine perfusion

3.1.1. Perfusion dynamics

Perfusion dynamics during HMP are shown in Fig. 1. There were no significant differences in overall perfusion dynamics during HMP between SCS4 and SCS52 kidneys. However the perfusion time necessary to reach viable perfusion parameters was significantly less in SCS52.

3.2. Normothermic reperfusion

3.2.1. Reperfusion dynamics

After 2 h of NRP reperfusion dynamics were not significantly different between SCS4 and SCS52 kidneys (see Fig. 2).

3.3. Functional assessment and tubular injury

Urine output during NRP was significantly higher in SCS4 kidneys (See Fig. 3). GFR demonstrated no significant difference between the two groups during NRP, however trended higher in SCS4 kidneys (See Fig. 4). Urinary LDH:GFR ratio demonstrated a similar level of tubular injury in both groups during NRP (see Fig. 5). Total NRP oxygen consumption was similar between both groups (See Fig. 3).

3.4. Rapid sampling microdialysis

Tissue lactate concentrations quantified using rsMD in kidneys during HMP (SCS4:n = 4, SCS52:n = 2) and NRP (SCS4:n = 2, SCS52:n = 2) and the real time profiles for each kidney can be seen in Fig. 4.

During HMP cortical lactate increased between 1.5 and 10 h in SCS4, p = 0.013. In contrast the lactate profiles for the SCS52 kidneys were variable and remained either stable or increased; with a

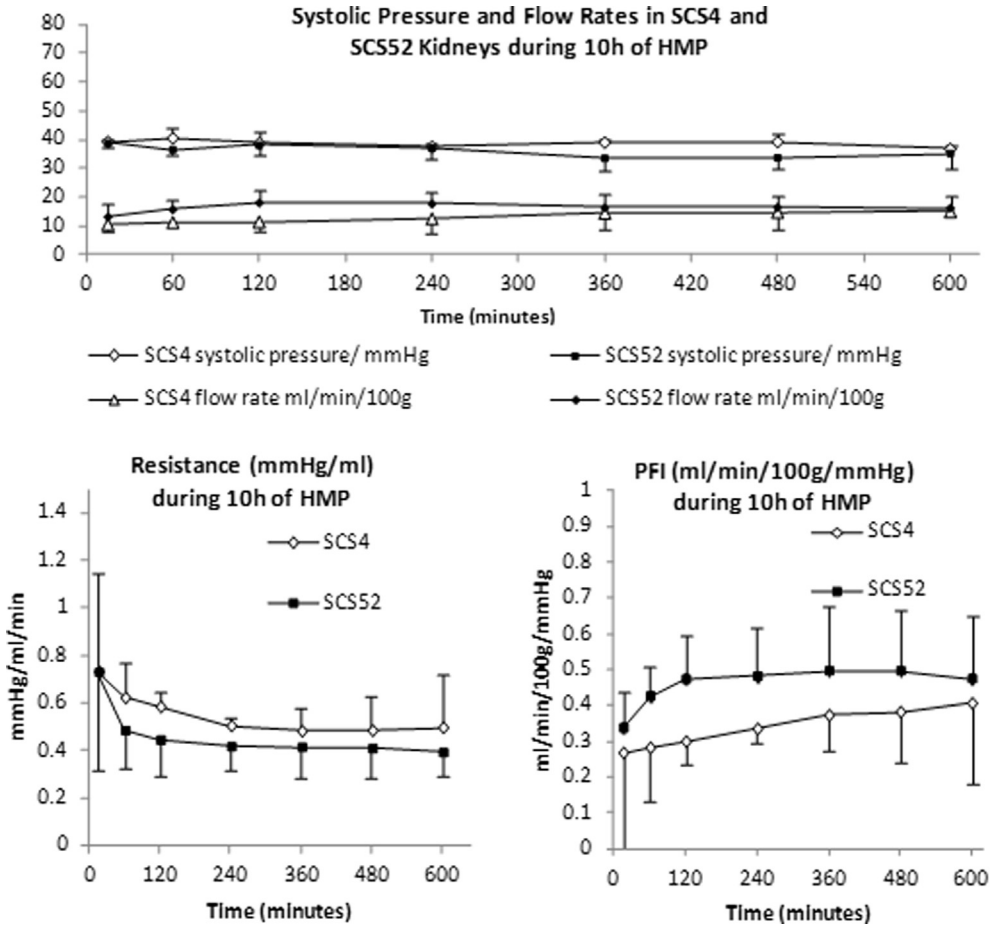

Fig. 1. Hypothermic machine perfusion dynamics in SCS4 and SCS52 kidneys. There were no significant differences in HMP perfusion parameters between kidneys. The perfusion time necessary to reach viable perfusion parameters (resistance<0.5 mmHg/ml/min, and PFI>0.4 ml/min/100 g/mmHg) was significantly less in SCS52 (Resistance: 165 ± 90 min, PFI: 120 ± 85 min) than SCS4 kidneys (Resistance: 450 ± 180 min, PFI: 330 ± 180 min; p = 0.008, p = 0.027 respectively).

trend of overall lower levels at 1.5 and then 10 h in SCS52 vs SCS4 kidneys.

During NRP lactate trended twofold higher in SCS4 kidneys (see Fig. 6 SCS4: 0.787 and 1.36 mM vs SCS52: 0.487 and 0.505 mM) and increased between the start and end of NRP in both groups. Statistical analysis of differences in lactate profiles was limited by the relatively small numbers in each group.

Cortical lactate correlated with perfusate lactate which also trended higher in SCS4 kidneys (SCS4 [n = 4]: 3.7 ± 0.39, SCS52 [n = 4]: 3.0 ± 0.69 mmol/L, p = 0.055) with the difference bordering significance.

3.5. Graft weight change

Kidney weight in the SCS4 group remained unchanged between flushing and the end of cold storage, while SCS52 kidneys lost weight (−17.1%, p = 0.012) after 52 h of cold storage. Weight change after HMP and NRP was not different between both groups (see Fig. 7).

3.6. Histology

There were no significant differences in histology between SCS4 and SCS52 kidneys pre-HMP and Post-HMP. After NRP all kidneys exhibited evidence of reperfusion induced tubular damage (tubular debris, tubular dilation, fragmented nuclei), and there were no significant differences between SCS4 and SCS52 kidneys, see Table 1 and Fig. 8.

4. Discussion

HMP reconditioning of kidneys after cold storage offers the possibility of re-vitalising allografts and metrics for viability assessment, especially in 'marginal' organs [12]. Characterising the potential for HMP reconditioning in DCD grafts exposed to long durations of cold storage is of particular interest for the successful expansion of the donor pool.

Porcine kidneys exposed to extreme SCS CIT demonstrated subsequent HMP dynamics suggestive of viability with equity to minimally injured grafts. HMP reconditioning however did not confer a functional advantage to kidneys with extreme SCS CIT during isolated NRP. Despite this, kidneys exposed to extremely long CITs demonstrated a similarity in alternative viability metrics (perfusion dynamics and oxygen consumption) during NRP to short length CIT kidneys.

4.1. Effect of long SCS on post-ischaemic HMP dynamics

HMP after a period of extreme SCS CIT in DCD porcine kidneys yields perfusion parameters indicative of suitable viability [13] for transplantation.

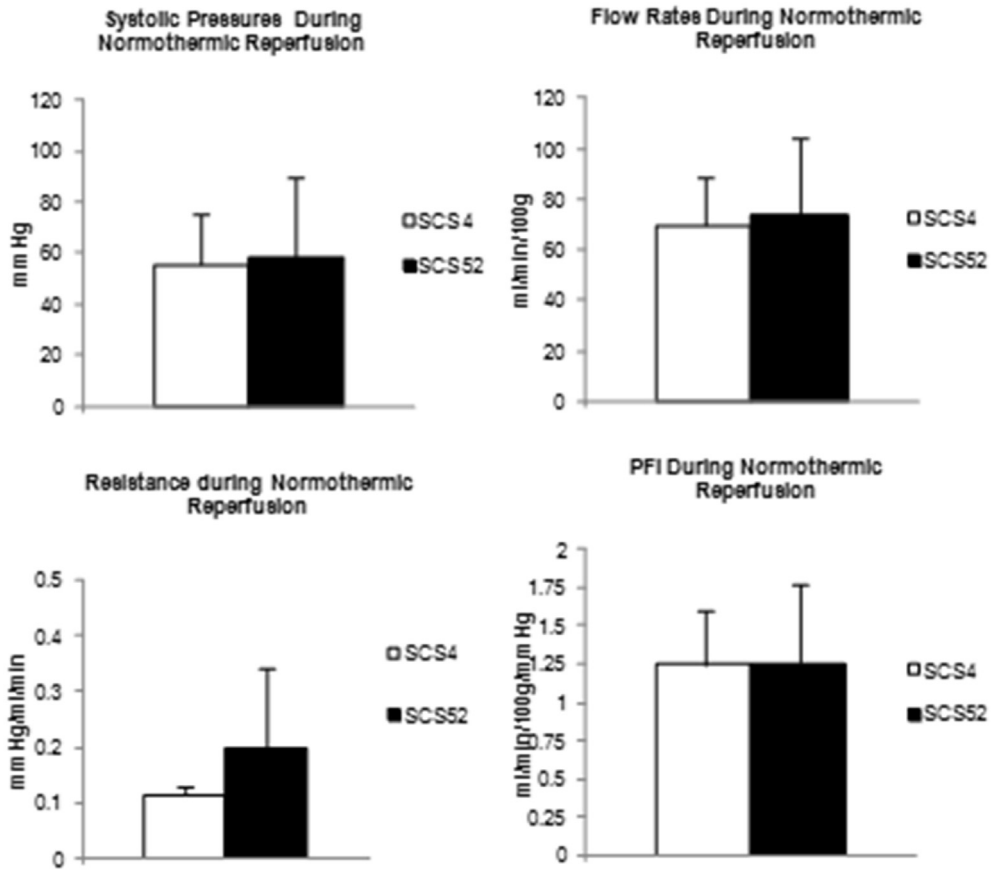

Fig. 2. Normothermic reperfusion dynamics in SCS4 and SCS52 kidneys. There were no significant differences in NRP parameters between kidneys. Systolic pressure: SCS4 = 55.7 ± 1.3, SCS52 = 58.1 ± 0.7 mmHg, p = 0.080; Reperfusion flow rate: SCS4 = 69.6 ± 19.64, SCS52 = 73.2 ± 31.6 ml/min/100 g, p = 0.853; Resistance: SCS4 = 0.11 ± 0.02, SCS52 = 0.19 ± 0.14 mmHg/ml/min, p = 0.305; PFI: SCS4 = 1.25 ± 0.36, SCS52 = 1.26 ± 0.53 ml/min/100 g/mmHg, p = 0.976.

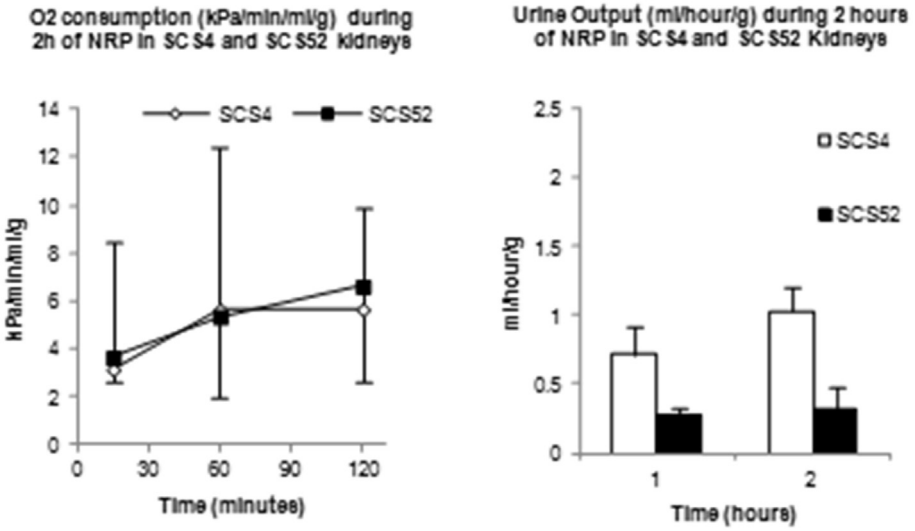

Fig. 3. Functional assessment in SCS4 and SCS52 kidneys. Urine output during NRP was significantly higher in SCS4 kidneys. Urine output 60 min: (SCS4 = 0.71 ± 0.21, SCS52 = 0.27 ± 0.06 ml/h/g, p = 0.034; Urine output 120 min: SCS4 = 1.03 ± 0.19, SCS52 = 0.32 ± 0.17 ml/h/g, p < 0.001). Oxygenation of the NRP perfusate resulted in a median arterial oxygen level of 22.6 7 ± 3.7 and 23.6 7 ± 7.3 kPa/min/ml/g in SCS4 and SCS52 kidneys respectively, with presence of oxygen in venous samples, indicating an adequate level of oxygenation of the perfusate during reperfusion. Total oxygen consumption during NRP was similar between SCS4 and SCS52 kidneys, SCS4: 6.1 ± 5.6, SCS52: 5.7 ± 2.5 kPa/min/ml/g, p = 0.894.

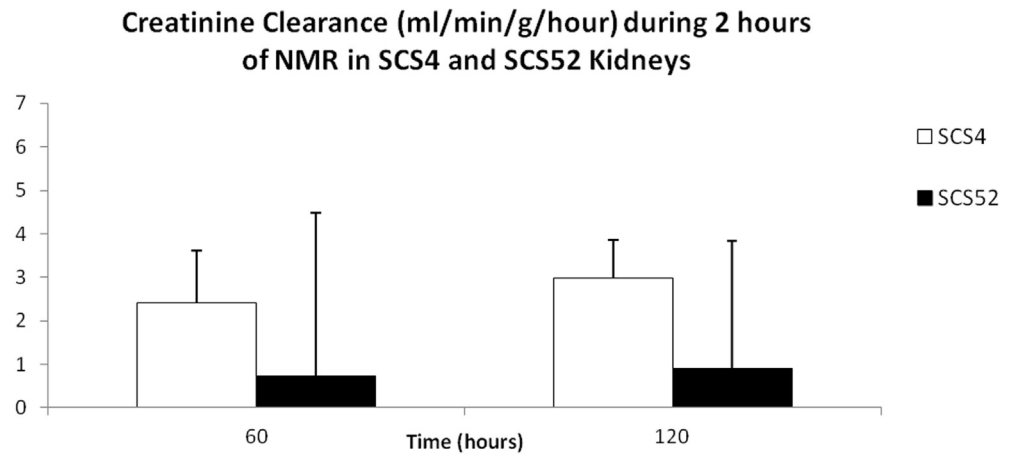

Fig. 4. Glomerular filtration rate during NRP in SCS4 and SCS52 kidneys at 60 min and 120 min. There was a non-significant trend of higher clearance rates in SCS4 vs SCS52 kidneys. SCS4: 2.41 ± 1.21 ml/min/g at 60 min, 3.0 ± 0.87 ml/min/g at 120 min; SCS52: 0.73 ± 3.76 ml/min/g at 60 min, 0.90 ± 2.94 ml/min/g at 120 min. Median±SD.

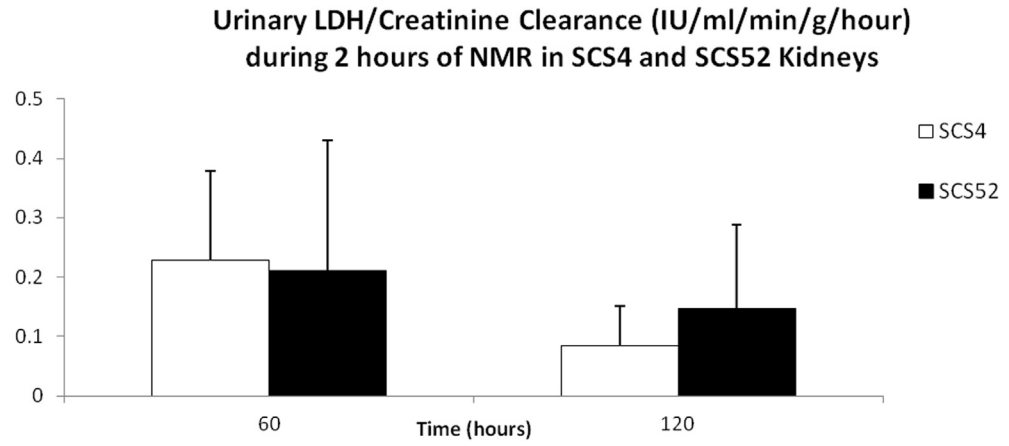

Fig. 5. Urinary LDH: GFR ratio for SCS4 and SCS52 kidneys during NRP at 60 min and 120 min of reperfusion. There was no difference between the two groups suggesting a similar degree of tubular injury (SCS4: 0.23 ± 0.15 IU/ml/min/g at 60 min, 0.08 ± 0.06 IU/ml/min/g at 120 min; SCS52: 0.21 ± 0.22 IU/ml/min/g at 60 min, 0.15 ± 0.14 IU/ml/min/g at 120 min) Median±SD.

Resistance and PFI during HMP are used by clinicians as measures of renal viability [13] and resistance has been shown to be an independent risk factor for primary non-function [14] and correlates with the post-transplant reperfusion renal resistivity index [17]. Perfusion dynamics are used by some centres to determine organ discard [15,16].

Overall HMP perfusion dynamics were similar in kidneys previously exposed to short SCS of 4 h and extreme SCS of 52 h. If clinical HMP viability criteria [13] (Resistance and PFI levels) were applied SCS52 kidneys attainted levels of accepted viability significantly earlier during HMP compared to SCS4 kidneys with shorter cold ischaemic injury. These findings were not expected.

Lower HMP flow rates, high resistance, and low PFI values are associated with poorer graft outcomes [18]. In this experiment the severely injured kidney grafts perfused as well and achieved 'viability' quicker as those with limited injury.

After extreme SCS (52 h) kidney weight decreased compared to weight at retrieval. Similar weight loss is reported after extended SCS of kidney allografts by other groups [19,20] and may be due to recovery from 'flush' induced oedema at retrieval, suggested to stem from malfunction of the Na+K+ pump, cellular swelling, and dehydration of the renal parenchyma during SCS [21]. The SCS4 kidneys with shorted SCS time did not experience a reduction in mass. The reduced oedema in SCS52 kidneys may have facilitated better perfusion during HMP with less tissue osmotic pressure resisting pulsatile flow. The SCS4 kidneys required several more hours of HMP to achieve optimal perfusion.

Lactate is the terminal product of anaerobic glycolysis; the primary source of ATP production during ischaemia [22]. Cortical cells are an endogenous source of lactate even during physiologically aerobic conditions [23]. These cells undergo anaerobic glycolysis to either augment low grade aerobic energy production during the passively oxygenated HMP, or to simply maintain physiological lactate production. The increasing trend in rsMD lactate during HMP suggests ongoing glycolytic metabolism, with the higher levels in SCS4 kidneys indicating potentially more viable cortical cells undergoing normal activity [11]. The lower levels in SCS52 kidneys likely reflects the extreme ischaemic damaged endured to those cells. Despite the lengthier damage however the data indicates that there are potentially viable cortical cells. The net weight loss observed in SCS52 kidneys prior to HMP which may represent more effective buffering and equilibration between the kidney tissue during the longer period of SCS.

Alternatively the observed viable dynamics during HMP of the SCS52 kidneys suggest that such perfusion metrics actually may not be reliable enough to differentiate between grafts with different

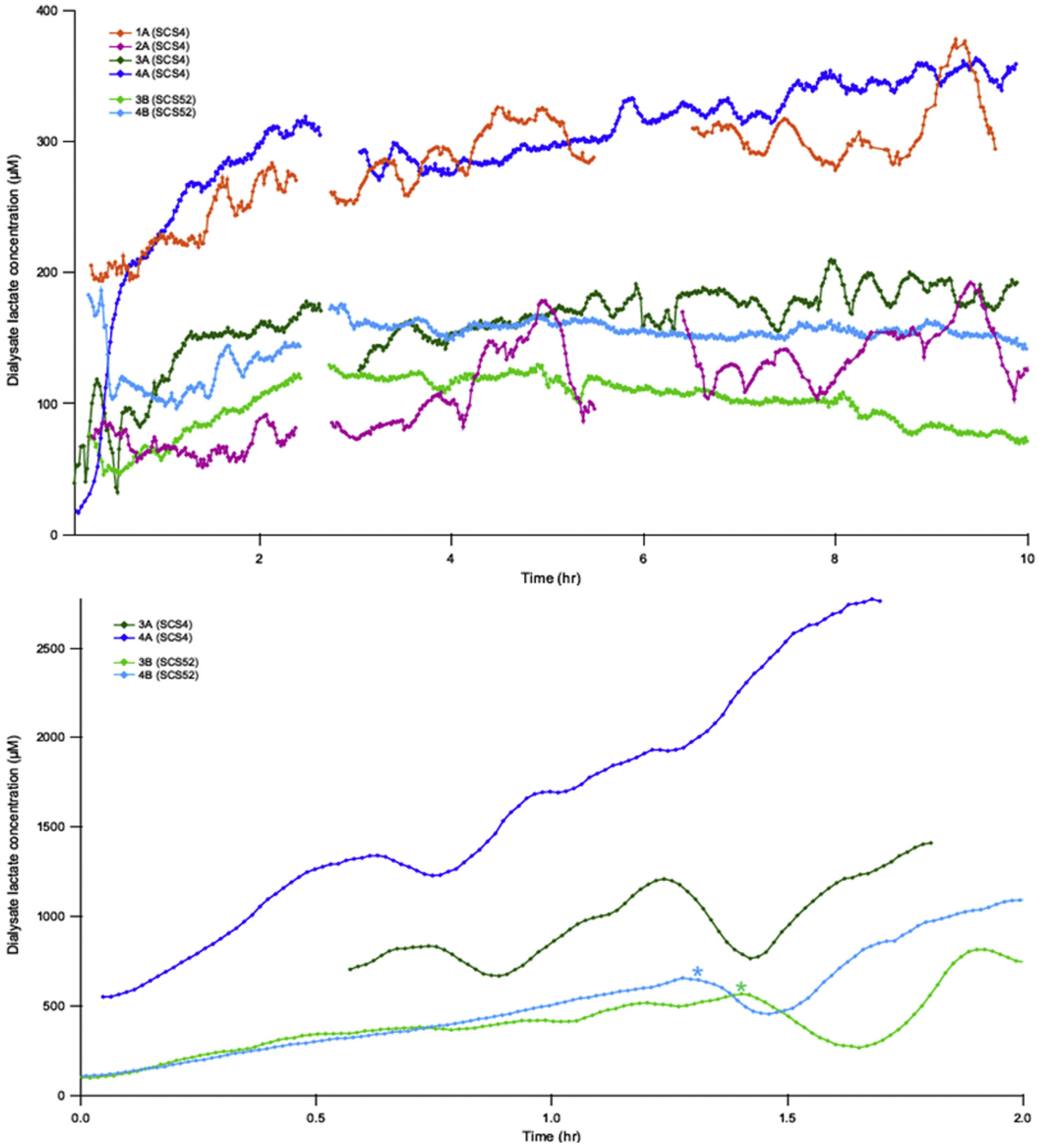

Fig. 6. RsMD profiles for tissue lactate during HMP (Top) and NRP (Bottom) in SCS4 and SCS52 kidneys. Each line represents an individual kidney. Top: Lactate in SCS4 kidneys progressively increased during 10 h of HMP (1.5 h: 0.193 ± 0.09 [Range: 0.064–0.264] mM, 10 h: 0.245 ± 0.104 [Range: 0.132–0.354] mM; p = 0.013). SCS 52 kidney lactate levels remained stable or increased (1.5 h: 0.081 and 0.111 mM, and 10 h: 0.080 and 0.158 respectively). During HMP SCS4 levels trended higher vs SCS52 levels (p > 0.05). Bottom: During NRP lactate increased between the start and end of NRP in both SCS4 and SCS52 kidneys (Start: 0.419 and 0.753 mM; End: 1.2 and 2.69 mM, change +0.784 and + 1.9435 mM respectively) and SCS52 kidneys (Start: 0.223 and 0.17 mM; End: 0.743 and 1.38, change +0.522 and + 1.208 mM respectively).

durations of previous CIT injury. In contrast interrogating tissue metabolism, quantifying cortical lactate levels does suggest the emergence of a potential difference between kidneys with different CITs and that rsMD could provide a more accurate assessment of organs during HMP preservation.

4.2. Effect of post-ischaemic HMP after extreme length of SCS on viability during reperfusion

During NRP SCS52 kidneys demonstrated equality in viability metrics compared to SCS4 kidneys. Both groups of kidneys

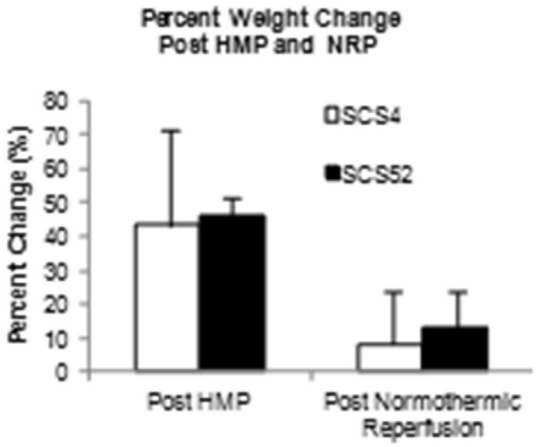

Fig. 7. Percent weight change in SCS4 and SCS52 kidneys during HMP and NRP. Weight change after HMP (SCS4: 43.1 ± 28.1%; SCS52: 46.3 ± 5.2%, p = 0.826) and NRP (SCS4: 8.1 ± 16.2%; SCS52: 13.4 ± 10.6%, p = 0.092) was not different between both groups of kidneys.

Table 1
Histology scores of biopsies taken after 2 h of NRP assessing tubular debris, tubular Dilatation, and nuclear fragmentation. There was no significant difference in the degree of tubular damage in SCS4 and SCS52 kidneys.

Parameter	Tubular Debris		Tubular Dilation		Nuclear Fragmentation	
Group	SCS4	SCS52	SCS4	SCS52	SCS4	SCS52
Score	3.5 ± 0.58	4.0 ± 0	2.3 ± 0.5	2.7 ± 0.6	2.1 ± 0.2	2.3 ± 0.3

perfused (flow and PFI) with equity during NRP, with comparable oxygen consumption, and similar GFRs, though there was a trend for lower filtration in the SCS52 kidneys, which was not unexpected considering the extreme length of cold ischaemia compared to the SCS4 group. However histology and urinary LDH:GFR suggested a similar degree of tubular injury in both groups.

rsMD observed higher overall cortical tissue lactate during NRP in kidneys exposed to only 4 h of SCS. During NRP lower levels were expected vs. the SCS52 kidneys, where full provision of oxygen would abort the need for anaerobic metabolism. In fact during IR a degree of anaerobic metabolism is necessary as the mitochondrial pathways required for aerobic ATP production are repaired [24]. Though raised tissue lactate is indicative of allograft thrombosis and tissue ischaemia, this correlation is accurate in a transplantation [25] or isolated haemoreperfusion model, where microcirculatory dysfunction can affect tissue perfusion and oxygen delivery. In this experiment with acellular KHB such microcirculatory failure is absent, and augmented cortical lactate production could suggest improved basal cortical cell viability [11].

The sustained and persistent production of cortical lactate by the SCS52 kidneys during NRP also suggests a degree of viability in the renal parenchyma of these kidneys which was not expected. This may be related to the initial finding of reduced post-ischaemic graft weight before HMP in those kidneys. A longer CIT that allows greater equilibration between the solution and the organ parenchyma may facilitate better preservation and buffering, which then allows improved HMP perfusion due to less graft oedema. Thus though shorter lengths of CIT are invariably more desirable and less damaging than longer CITs, a longer CIT may confer a better SCS preservation effect from the solution. The longer-term effect of this better equilibration however on renal function is unknown but the possibility that kidneys initially considered to be extremely marginal due to lengthy cold ischaemia may actually be useable after a period of post-ischaemic HMP deserves further investigation.

4.3. Mechanisms of effects of post-ischaemic HMP

Despite similar viability metrics during NRP, 10 h of HMP was not sufficient to restore renal function. Oxygenation during HMP may be a potential factor. Recent experimental evidence has suggested that oxygenation during HMP may be potentially beneficial and restore cellular energy stores and improve allograft function during reperfusion [4] though this may be limited to specific donor types [26]. In our study passive oxygenation with room air on a RM3 was used during HMP as in clinical practice, this provides a perfusate oxygen pressure of approximately 24 kPa (180 mmHg) [27] which is theoretically sufficient for tissue needs at 4–7 °C [28,29] sustaining kidney metabolism. Active oxygenation delivering a higher partial pressure of oxygen may however afford further benefit in kidneys that have sustained severe ischaemic stress, such as those in the SCS52 group.

4.4. Limitations

There are several caveats to the interpretation of this study. The HMP dynamics and effects on HMP on kidney viability in the SCS4 group was considered in this model to reflect ideal kidneys with minimal CIT injury, and a suitable comparison for kidneys exposed to prolonged SCS prior to HMP. The next evolution of these experiments would investigate the benefit of a period of HMP or not after exposure to extreme SCS CIT.

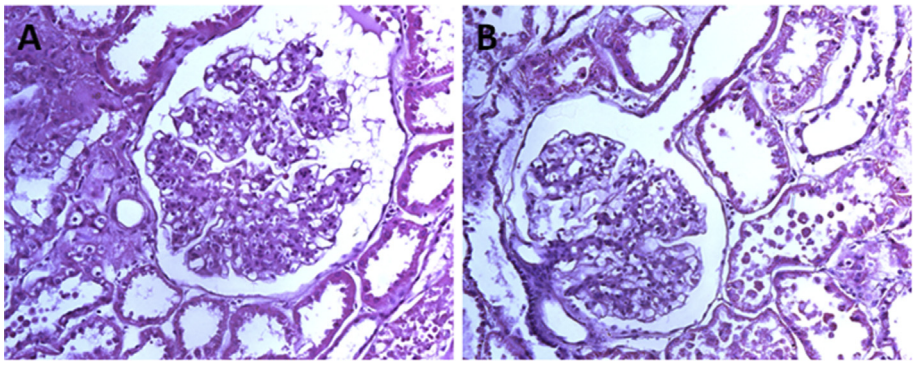

Fig. 8. Photomicrographs of light microscopy of SCS4 vs. SCS52 kidneys after 2 h of NRP. H&E staining, ×20. A: SCS4 kidney, B: SCS52 kidney. Showing similar degrees of reperfusion injury.

Due to technical problems with the rsMD hardware/software interface data was not available for all kidneys during both HMP and NRP (available for 6 kidneys during HMP, 4 during NRP) this limited significance in observed temporal differences in kidneys during HMP, and then during NRP, as well as differences between the SCS4 and SCS52 kidneys. Perfusate lactate levels however were successfully measured for all 8 kidneys and demonstrated a trend similar to the rsMD data that was available to support those observations.

NRP using acellular KHB is useful for acute determination of renal function and viability, however isolated haemoreperfusion would provide a more physiological assessment and challenge in kidney grafts [29].

rsMD concentrated on lactate as a marker of ischaemic injury, however detection of other glycolytic molecules such as glucose, pyruvate and ATP will provide a greater assessment of tissue metabolism and sensors to detect these metabolites are in development.

Finally HMP was only passively oxygenated as per clinical practice with the RM3 unit. There is however a potential benefit of actively oxygenated HMP after extreme SCS on renal viability which is another avenue of investigation.

5. Conclusion

As post-ischaemic HMP reconditioning emerges as a viable technique for revitalising marginal allografts [1] consideration must be made as to the length of SCS from which the allograft is being recovered. In our study porcine kidneys exposed to extreme SCS CIT demonstrated subsequent HMP dynamics suggestive of clinical viability with equity to minimally injured grafts, while the use of a novel microdialysis system to detect cortical tissue lactate levels did suggest subtle differences in kidney viability and metabolism during dynamic preservation. During normothermic reperfusion kidneys demonstrated similar viability metrics (perfusion dynamics, GFR, oxygen consumption, urinary LDH:GFR, histology) as kidneys with shorter CIT. These findings may relate to the observed graft weight loss during extremely long SCS, where resolution of flush induced oedema through better equilibration with the solution during cold storage may facilitate better perfusion dynamics during subsequent HMP and graft re-conditioning. The relevance of these improved dynamics in relation to tissue viability is thus uncertain. It will be important for practitioners of HMP to be aware that graft weight loss during SCS, and hence resolution of flush induced oedema may affect perfusion dynamics during HMP. There is potential for use of microdialysis as a novel adjunct in organ assessment during preservation determining renal cortical metabolism, and differences in kidney viability and sustained injury. Finally despite the clinical success with conventional HMP, in its present form utilising passive oxygenation, HMP may not be able to functionally recover grafts exposed to extreme SCS, suggesting that HMP interventions utilising active oxygenation of the perfusate may be better positioned to provide benefit in such organs.

Ethical approval

This research was conducted at laboratories of Imperial College London. Local guidelines and policies were adhered to regarding the use of animal tissue. No specific ethics approval was required.

Funding

This research was funded by grants from the Live Life Give Life, UK.

Author contribution

Authors:

Karim Hamaou, Sally Gowers, Bynvant Sandhu, Terry Cook, Martyn Boutelle, Daniel Casanova-Rituerto, Vassilios Papalois.

Authorship:

KH engaged in research design, performance of the work, data analysis and writing the paper. SG and BS participated in performance of the work and data analysis. DC participated in research design and performance of the research. MB and TC participated in data analysis and contributed analytical expertise and tools. VP participated in research design, data analysis and writing the paper. All authors contributed to reviewing the manuscript.

Conflict of interest statement

None.

Guarantor

Karim Hamaoui.
Vassilios Papalois.

Research Registration Number

NA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijso.2019.10.002.

References

[1] Minor T, Paul A. Hypothermic reconditioning in organ transplantation. Curr Opin Organ Transplant 2013;18:161–7.
[2] Koetting M, Minor T. Donation after cardiac death: dynamic graft reconditioning during or after ischemic preservation? Artif Organs 2011;35:565–71.
[3] Hamaoui K, Gowers S, Boutelle M, Cook TH, Hanna G, Darzi A, et al. Organ pretreatment with cytotopic endothelial localizing peptides to ameliorate microvascular thrombosis and perfusion deficits in ex vivo renal hemoreperfusion models. Transplantation 2016;100:e128–39.
[4] Koetting M, Frotscher C, Minor T. Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. Transpl Int 2010;23:538–42.
[5] Gallinat A, Paul A, Efferz P, Lüer B, Kaiser G, Wohlschlaeger J, et al. Hypothermic reconditioning of porcine kidney grafts by short-term preimplantation machine perfusion. Transplantation 2012;93:787–93.
[6] Treckmann J, Moers C, Smits JM, Gallinat A, Maathuis M-HJ, van Kasterop-Kutz M, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. Transpl Int 2011;24:548–54.
[7] Watson CJE, Wells AC, Roberts RJ, Akoh JA, Friend PJ, Akyol M, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. Am J Transplant 2010;10:1991–9.
[8] Ciancio G, Gaynor JJ, Sageshima J, Roth D, Kupin W, Guerra G, et al. Machine perfusion following static cold storage preservation in kidney transplantation: donor-matched pair analysis of the prognostic impact of longer pump time. Transpl Int 2012;25:34–40.
[9] Wilson CH, Gok MA, Shenton BK, Balupuri S, Gupta AJ, Asher J, et al. Weight increase during machine perfusion may be an indicator of organ and in particular, vascular damage. Ann Transplant 2004;9:31–2.
[10] Hamaoui K, Aftab A, Gowers S, Boutelle M, Cook T, Rudd D, et al. An ex vivo comparison of adenosine and lidocaine solution and University of Wisconsin solution for hypothermic machine perfusion of porcine kidneys: potential for development. J Surg Res 2017;208:219–29.
[11] Hamaoui K, Gowers S, Damji S, Rogers M, Leong CL, Hanna G, et al. Rapid sampling microdialysis as a novel tool for parenchyma assessment during static cold storage and hypothermic machine perfusion in a translational ex vivo porcine kidney model. J Surg Res 2015;1–14.

[12] Gallinat A, Moers C, Smits JM, Strelniece A, Pirenne J, Ploeg RJ, et al. Machine perfusion versus static cold storage in expanded criteria donor kidney transplantation: 3-year follow-up data. Transpl Int 2013;26:E52–3.
[13] Talbot D, Shenton B, Buckley P. Experiences learned in the successful establishment of a nonheart beating donor program for renal transplantation. J Urol 2003;170:1088–92.
[14] de Vries EE, Hoogland ERP, Winkens B, Snoeijs MG, van Heurn LWE. Renovascular resistance of machine-perfused DCD kidneys is associated with primary nonfunction. Am J Transplant 2011;11:2685–91.
[15] Patel SK, Pankewycz OG, Nader ND, Zachariah M, Kohli R, Laftavi MR. Prognostic utility of hypothermic machine perfusion in deceased donor renal transplantation. Transplant Proc 2012;44:2207–12.
[16] Jochmans I, Moers C, Smits JM, Leuvenink HGD, Treckmann J, Paul A, et al. The prognostic value of renal resistance during hypothermic machine perfusion of deceased donor kidneys. Am J Transplant 2011;11:2214–20.
[17] Munteanu V, Moga S, Suciu M, Enache DAN, Elec A, Munteanu A, et al. Ex-vivo perfusion machines in kidney transplantation. The significance of the resistivity index. Clujul Med 2014;87:27–9.
[18] Balfoussia D, Yerrakalva D, Hamaoui K, Papalois V. Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. Exp Clin Transplant 2012;10:87–100.
[19] Hosgood SA, Mohamed IH, Bagul A, Nicholson ML. Hypothermic machine perfusion after static cold storage does not improve the preservation condition in an experimental porcine kidney model. Br J Surg 2011;98:943–50.
[20] Vaziri N, Thuillier RR, Favreau FD, Eugene M, Milin S, Chatauret NP, et al. Analysis of machine perfusion benefits in kidney grafts: a preclinical study. J Transl Med 2011;9:15.
[21] La Manna G, Conte D, Cappuccilli ML, Nardo B, D'Addio F, Puviani L, et al. An in vivo autotransplant model of renal preservation: cold storage versus machine perfusion in the prevention of ischemia/reperfusion injury. Artif Organs 2009;33:565–70.
[22] Kallerhoff M, Blech M, Kehrer G, Kleinert H, Langheinrich M, Siekmann W, et al. Effects of glucose in protected ischemic kidneys. Urol Res 1987;15:215–22.
[23] Bellomo R. Bench-to-bedside review: lactate and the kidney. Crit Care 2002;6:322–6.
[24] Silva MA, Murphy N, Richards DA, Wigmore SJ, Bramhall SR, Buckels JAC, et al. Interstitial lactic acidosis in the graft during organ harvest, cold storage, and reperfusion of human liver allografts predicts subsequent ischemia reperfusion injury. Transplantation 2006;82:227–33.
[25] Keller AK, Jørgensen TM, Vittrup DM, Kjerkegaard UK, Jespersen B, Krag SRP, et al. Fast detection of renal ischemia in transplanted kidneys with delayed graft function-an experimental study. Transplantation 2013;95:275–9.
[26] Gallinat A, Paul A, Efferz P, Lüer B, Swoboda S, Hoyer D, et al. Role of oxygenation in hypothermic machine perfusion of kidneys from heart beating donors. Transplantation 2012;94(8):809–13.
[27] Lindell SL, Muir H, Brassil J, Mangino MJ. Hypothermic machine perfusion preservation of the DCD kidney: machine effects. J Transplant 2013;2013:802618.
[28] Guibert EE, Petrenko AY, Balaban CL, Somov AY, Rodriguez JV, Fuller BJ. Organ preservation: current concepts and new strategies for the next decade. Transfus Med Hemotherapy 2011;38:125–42.
[29] Mangino MJ, Lindell SL. Hypothermic machine perfusion of kidneys. In: Lee CY, Uygun K, editors. Methods bioeng. Organ preserv. Reengineering. Norwood, MA: Artech House; 2011. p. 35–57.