Chapter 2

Comparison of preservation solutions for washout of kidney grafts: an experimental study
Comparison of preservation solutions for washout of kidney grafts: an experimental study

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Abstract

Objective
The impact of different preservation solutions for washout of kidney grafts was evaluated regarding temperature, kidney weight, remaining red blood cells (RBCs) and histological evaluation after ex vivo washout using 500 mL cold preservation solution at 4°C followed by 24 hours cold storage (CS).

Methods
Kidneys retrieved from Landrace pigs (20-30 kg) were immediately washed (warm ischemic time 0 min [WIT 0]), using 500 mL cold University of Wisconsin solution (UW), histidine-tryptophan-ketoglutarate (HTK), or Polysol (PS) followed by 24 hours CS. Also, kidneys were retrieved after a WIT of 30 minutes followed by washout using HTK or PS.

Results
After washout, the weight of kidneys washed out with HTK had increased, whereas that of organs in the UW or PS group had decreased. After washout with UW, the core temperature of WIT 0 kidneys was lower than that with HTK. The time needed for washout using 500 mL solution was shorter using PS compared with HTK for both WIT 0 and WIT 30 groups. The amount of remaining RBCs was similar between all WIT 0 groups; whereas in the WIT 30 groups the amount was higher in kidneys washed out using HTK compared with PS. Histological evaluation showed less tissue injury among PS-washed kidneys compared with UW or HTK.

Conclusion
Overall, kidneys washed-out with PS showed better preservation of structural integrity after 24 hours CS compared with either UW or HTK. Washout of warm ischemically damaged kidneys was more effective using PS compared with HTK.
Introduction

The quality of organ preservation is generally considered to be a major determinant of graft function and graft survival. The period of organ preservation starts at procurement by rapidly cooling the graft using precooled solution. The temperature of the graft must be reduced as rapidly as possible to reduce graft metabolism and to attenuate preservation injury. Adequate tissue equilibrium of the graft due to homogeneous perfusion by the preservation solution is considered to be a prerequisite for maintenance of graft viability. The graft is also washed out to remove blood remnants, seeking to prevent occlusion of the vascular bed before ischemic cold storage (CS). Incomplete graft washout not only prevents the tissue from being protected by the preservation solution, but also the presence of blood remnants and cellular debris may contribute to impaired blood flow upon reperfusion. In liver transplantation, insufficient graft perfusion during washout can lead to ischemic-type biliary lesions. The viscosity of the preservation solution plays an important role in its ability to effectively rinse the organ. Preservation solutions must decrease the graft’s temperature as rapidly as possible to provide optimal protection during storage. An effective washout and homogeneous distribution of the preservation solution in the graft prevents cell swelling, formation of interstitial edema, and excessive cellular acidosis.

The present study sought to evaluate the efficacy of graft cooling and red blood cell washout as well as the quality of CS preservation using currently applied solutions for organ procurement: We compared the washout and CS of heart-beating donor (HBD) porcine kidneys using the University of Wisconsin solution (UW), Histidine-Tryptophan-Ketoglutarate solution (HTK), and Polysol solution (PS) (Table 1). In addition, we compared the efficacy of washout and subsequent CS of warm ischemically damaged kidney grafts using PS and HTK, which owing to its low viscosity has become the standard preservation solution for non-heart-beating donor organs.

Materials and methods

Animals and experimental protocols

All experiments were performed in accordance with German legislation governing animal studies following the *Principles of Laboratory Animal Care* (National Institutes of Health publication 85-23, revised 1985). Kidneys were retrieved from female German landrace pigs, weighing 26.0 ± 3.0 kg (mean ± SD). This study included two donor categories; organs from heart-beating donors (WIT 0) and those subjected to a warm ischemic time (WIT) of 30 minutes (WIT 30).
Surgical Procedures
Ten minutes before induction of anesthesia, animals were premedicated with ketamine (90 mg/kg), xylazine (10 mg/kg) and atropine (0.01 mg/kg) administered intramuscularly. General anesthesia was induced by midazolam (0.5 mg/kg), and fentanyl (12.5 μg/kg), muscle relaxation was achieved by pancuronium (0.2 mg/kg), all administered intravenously (IV) by means of a butterfly cannula inserted into an external marginal ear vein. The animals were maintained after intubation by mechanical ventilation with isoflurane. Thereafter, the right internal jugular vein was cannulated with a polyethylene (PE) catheter for infusion. Via laparotomy, we performed a nephrectomy. Animals were killed by injection of T61 IV (Hoechst Roussel Vet, Wiesbaden, Germany).

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<tr>
<th>Table 1. Composition of preservation solutions used</th>
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<td>Viscosity at 5°C (cP)</td>
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UW, University of Wisconsin solution; HTK, Histidine-Tryptophan-Ketoglutarate; PS, Polysol; HES, hydroxyethyl starch; PEG, polyethylene glycol; cP, centi-Poise.  
<sup>a</sup>The following amino acids are present in Polysol: alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine.

<sup>b</sup>The following vitamins are present in Polysol: ascorbic acid, biotin, Ca-pantothenate, choline chloride, inositol, ergocalciferol, folic acid, menadione, nicotinamide, nicotinic acid, pyridoxal, riboflavin, thiamine, vitamin A, vitamin B12 and vitamin E.

Heart-beating donor organs
This study compared washout of heart-beating donor kidneys using UW (Viaspan; Bristol-Myers Squibb, Woerden, The Netherlands), HTK
(Custodiol; Dr Franz Köhler Chemie, Alsbach-Hänlein, Germany), or PS solution (Doorzand Medical Innovations, Amsterdam, The Netherlands) followed by 24 hours CS in the respective solution (Table 1). Twenty-four hours before washout of the grafts, perfusates were placed in a microcomputer-controlled cold water bath at 4°C (Lauda, Königshofen, Germany). After retrieval, the kidneys \((n=5\) in each group) were weighed followed by an immediate ex vivo washout using 500 mL cold preservation solution (4°C) at a hydrostatic pressure of 100 cm H\(_2\)O via the renal artery. During washout, the core temperature of the kidneys was assessed every 30 seconds using a digital handheld thermometer with two temperature probes (Fluke 54 II thermometer; Fluke Deutschland, Kassel, Germany). One probe which measured the core temperature of the kidney was inserted approximately 10 mm into the kidney parenchyma just before washout; the other probe measured the ambient temperature. We also recorded the time to flush the kidney with 500 mL preservation solution. After washout, the kidneys were weighed and placed in a sterile bag with 500 mL fresh preservation solution for cold storage at 4°C using a cold water bath (Lauda, Königshofen, Germany) for 24 hours. After preservation, kidneys were weighed and biopsies obtained for histological analysis.

Warm ischemically damaged donor organs

Before retrieval, the kidneys were subjected to a warm ischemic time of 30 minutes (WIT 30) by clamping the renal pedicle. Then they were weighed, directly followed by an ex vivo washout using 500 mL cold HTK (HTK WIT 30; \(n=7\)) or PS (PS WIT 30, \(n=7\)) at a hydrostatic pressure of 100 cm H\(_2\)O (4°C) via the renal artery. We assessed the core temperature of the kidneys every 30 seconds and the times to flush the kidneys with 500 mL cold preservation solution. Subsequently, the kidneys placed in a sterile bag with 500 mL either HTK or PS were stored at 4°C for 24 hours. Thereafter, tissue specimens were obtained for histological evaluation.

Histological Analysis

Via light microscopy kidneys fixed in neutral 10% buffered formalin and embedded in paraffin were examined in sections for the anatomy of the cortex, medulla, and corticomedullary junction.

Red blood cell count

For a quantitative evaluation of the washout, the remaining red blood cells (RBCs) were counted in specimens of the corticomedullary junction. In a blinded manner, counting of RBCs was performed in ten randomly selected fields of hematoxylin and eosin (H&E)-stained sections\(^\text{10}\).
Renal morphological studies
Tissue injury was assessed on periodic acid-Schiff (PAS)-stained 2-µm-thick sections by scoring glomerular shrinkage, tubular damage, interstitial edema, and necrosis. Injury in each specimen was graded to the extent of regional involvement in ten randomly chosen non-overlapping fields (original magnification X400). Injury was scored by a pathologist blinded for the groups using a 5-point scale: 0 = no damage; 1 = lesions affecting ≤10% of the field; 2 = 10%-25%; 3 = 25%-50%; 4 = 50%-75% and 5 = >75%.11

Statistics
Results are expressed as mean values ± SD. For the HBD series, we used one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Statistical analysis of kidney weights was performed using repeated-measurements (RM) ANOVA. Remaining RBCs were statistically evaluated using the Kruskal-Wallis test. For the series on warm ischemically damaged kidneys, we performed the unpaired two-tailed Student t-test. For nonparametric examinations, we applied the Mann–Whitney test. Areas under the curve (AUC) for core temperature during washout were calculated individually using the GraphPad Prism 5.0 statistics package (GraphPad Software, San Diego, CA, USA). A P-value of < .050 was considered to be significant.

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<th>Table 2. Kidney weights (g) at different time points during the experiment</th>
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<th>Warm ischemically damaged kidneys (WIT 30)</th>
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<td>pre-washout</td>
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<td>post-washout</td>
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<td>post 24 h CS</td>
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WIT 0, warm ischemic time 0 min; WIT 30, warm ischemic time 30 min, NS, not significant, Data expressed as mean ± SD. **P < .010, ***P < .001.
†Repeated-measurements ANOVA followed by Bonferroni posttest.
Results

Heart-beating donors
The body weights of the pigs did not differ between the groups: UW WIT 0 24.6 ± 2.9 kg, HTK WIT 0 25.9 ± 2.3 kg, and PS WIT 0 25.5 ± 4.3 kg (P = .846). Kidney weights before washout were similar between the groups (Table 2).

Repeated-measurement analyses for kidney weights before washout, after washout, and after 24 hours CS demonstrated similar kidney weights between the groups before washout and after 24 hours CS. After washout, kidney weights were higher among the HTK WIT 0 group compared with either the UW WIT 0 or the PS WIT 0 group (P = .073; HTK WIT 0 versus UW WIT 0: P < .001; HTK WIT 0 versus PS WIT 0: P < .01; Table 2).

At the beginning of the washout, core kidney temperatures did not differ between the groups: UW WIT 0, 36.3 ± 0.4°C; HTK WIT 0, 34.6 ± 2.1°C; PS WIT 0, 35.9 ± 0.6°C (P = .764). Overall, core temperatures of washed-out kidneys using 500 mL cold UW were lower compared with those using HTK: two-way RM ANOVA: UW WIT 0, 9.7 ± 2.4°C; HTK WIT 0, 12.9 ± 0.2°C; PS WIT 0, 10.9 ± 1.2°C (HTK WIT 0 versus UW WIT 0: P < .01). There was no difference between the UW WIT 0 and PS WIT 0 group.

Warm ischemically damaged donor organs
The body weight of the pigs did not differ between the groups (HTK WIT 30, 25.7 ± 3.8 kg; PS WIT 30, 27.4 ± 2.0 kg; (P = .338). Also, before washout there was no difference in kidney weights. After HTK washout, the kidneys had gained significantly more weight than the warm ischemically damaged kidneys washed out using PS: HTK WIT 30 plus 34.8 ± 8.5% and PS WIT 30 minus 0.8 ± 3.6% (P = .002). After 24 hours CS, the weights in both groups were reduced and overall did not differ between the groups: RM ANOVA for kidney weights before washout, after washout, and after 24 hours CS (P = .439; Table 2).

At the beginning of the washout, the core temperatures of kidneys were similar: HTK WIT 30, 34.3 ± 1.5°C; PS WIT 30, 35.5 ± 1.0°C (P = .124). After washout, the core temperature of PS-washed out kidneys was lower than that of organs after HTK: HTK WIT 30, 14.9 ± 1.5°C; PS WIT 30, 12.2 ± 1.6°C (P = .01). Also, the times required for washout with 500 mL preservation solution were shorter using PS compared with HTK: HTK
WIT 30, 18.3 ± 4.6 min; PS WIT 30, 12.8 ± 2.4 min (P = .021). The AUC for the core temperatures of the kidneys was lower in the PS WIT 30 group than in the HTK WIT 30 group: HTK WIT 30, 306.5 ± 22.1; PS WIT 30, 222.9 ± 26.7 (P = .033; Fig. 1B).

**Fig. 1.** Kidney core temperatures during washout. **(A)** Heart beating donor kidneys (WIT 0), core temperature of kidneys during washout using UW, HTK, or PS solution. * AUC UW WIT 0 versus HTK WIT 0 and PS WIT 0 versus HTK WIT 0: P < .050. **(B)** Warm ischemically damaged kidneys (WIT 30), core temperature of kidneys during washout using HTK or PS solution. * AUC PS WIT 30 versus HTK WIT 30: P < .050.
Red blood cell count
After washout with 500 mL of preservation solution, all WIT 0 groups showed a macroscopically homogeneous washout with a comparable amount of remaining RBCs (Fig. 2).

Fig. 2. (A) Remaining red blood cells after washout and 24 h CS of porcine heart beating donor kidneys: UW WIT 0, 243.3 ± 38.2; HTK WIT 0, 225.5 ± 65.2; PS WIT 0, 141.8 ± 47.6; P = .055 (data expressed as mean ± SD). (B-D) Light microscopy, representative histological pictures of H&E-stained sections per group (magnification X40). Arrows show remaining red blood cells. (B) UW WIT 0 group, (C) HTK WIT 0, (D) PS WIT 0.
Macroscopic evaluation of the washout in the WIT 30 groups showed heterogeneous washout of kidneys using HTK as opposed to a homogeneous washout in the PS group. This subjective finding was confirmed by the amount of RBCs remaining in the kidney after washout: HTK WIT 30, 217.6 ± 66.3; PS WIT 30, 75.7 ± 45.0 ($P = .0006$; Fig. 3).

Fig. 3. (A) Red blood cell count after washout and 24 h CS of porcine warm ischemically damaged kidneys: HTK WIT 30, 217.6 ± 66.3; PS WIT 30, 75.7 ± 45.0; $P = .0006$ (data expressed as mean ± SD). (B,C) Light microscopy, representative histological pictures of H&E-stained sections per group (magnification X400). Arrows show remaining red blood cells. (B) HTK WIT 30, (C) PS WIT 30.
Renal morphology studies
In the WIT 0 groups, histological evaluation of kidneys after washout followed by 24 hours’ CS showed less interstitial edema with PS- compared with UW- or HTK-preserved kidneys. Also, less necrosis was observed in the PS WIT 0 group compared with the HTK WIT 0 group (Table 3). Overall, the structural integrity was better preserved in the PS WIT 30 group compared with the HTK WIT 30 group (Table 3).

### Table 3. Histological analysis

#### Heart-beating donor kidneys (WIT 0)

<table>
<thead>
<tr>
<th></th>
<th>UW WIT 0 (n = 5)</th>
<th>HTK WIT 0 (n = 5)</th>
<th>PS WIT 0 (n = 5)</th>
<th>P-value†</th>
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<tbody>
<tr>
<td>Glomerular damage</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.5</td>
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<tr>
<td>Tubular damage</td>
<td>2.2 ± 0.4</td>
<td>2.0 ± 0.5</td>
<td>2.1 ± 0.2</td>
<td>0.279</td>
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<tr>
<td>Interstitial edema</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.2</td>
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<td></td>
<td>UW WIT 0 vs PS WIT 0 **</td>
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<td></td>
<td>HTK WIT 0 vs PS WIT 0 *</td>
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<tr>
<td>Necrosis</td>
<td>0.3 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.1 ± 0.3</td>
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<tr>
<td></td>
<td>HTK WIT 0 vs PS WIT 0 ***</td>
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#### Warm ischemically damaged kidneys (WIT 30)

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<tr>
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<th>HTK WIT 30 (n = 7)</th>
<th>PS WIT 30 (n = 7)</th>
<th>P-value‡</th>
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<tr>
<td>Glomerular damage</td>
<td>3.0 ± 0.4</td>
<td>1.3 ± 0.7</td>
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<tr>
<td>(shrinking)</td>
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<tr>
<td>Tubular damage</td>
<td>3.3 ± 0.8</td>
<td>1.8 ± 0.2</td>
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<tr>
<td>Interstitial edema</td>
<td>2.7 ± 0.4</td>
<td>1.8 ± 0.3</td>
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<tr>
<td>Necrosis</td>
<td>1.1 ± 0.8</td>
<td>0.3 ± 0.3</td>
<td>.0026</td>
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Semiquantitive scale: 0 = no abnormality; 1 = mild, lesions affecting 0%-10% of the field; 2 = moderate, lesions affecting 10%-25% of the field; 3 = severe, lesions affecting 25%-50% of the field; 4 = very severe, lesions affecting 50%-75% of the field; 5 = extensive damage, involvement of > 75% of the field. Data expressed as mean ± SD, *P < .05, **P < .001, † Kruskal-Wallis test followed by Dunn multiple comparison, ‡ Mann-Whitney test.
Discussion

We performed a comparative analysis of the washout efficacy of various preservation solutions for kidney grafts. In the Eurotransplant region, macroscopically determined suboptimal washout is considered to be a reason for refusal of a graft. Moreover, it is commonly accepted that the outcome of transplantation is influenced by an effective washout of blood remnants and cellular debris as well as the length of the WIT. Kubota et al. noted that WIT is an independent risk factor for renal graft survival. Immediate cooling of the graft to slow down metabolism is therefore an essential step in maintaining viability during the ischemic period of storage before transplantation. In clinical transplantation, the use of HTK solution in liver transplantation has initially been described to involve high volume (10-15 L) compared with the low-volume washout of liver grafts using UW (3-5 L). More recently, however, Mangus et al. described equivalent clinical outcomes after liver transplantation when using 3-4 L HTK solution. For reasons of standardization, in the present study we compared the impact of different preservation solutions during washouts using equal volumes (500 mL). Our data suggested that cooling of kidney grafts was more effective using UW or PS compared with HTK. Kidney washout with 500 mL HTK, in both WIT 0 and WIT 30 groups with HTK took longer than with PS. Also, the core temperature of kidneys washed out with HTK did not reach temperatures below 12°C, whereas those washed out using UW or PS resulted in core temperatures between 9°C and 12°C. The rate of metabolism, though reduced, is still active at temperatures below 10°C, with less metabolic processes remaining active as the temperature is reduced. During ischemia, the core temperature and tissue metabolism should therefore be reduced as soon and as much as possible. Apart from the importance of rapid effective cooling of the graft, a complete washout of donor blood from and a homogeneous distribution of the preservation solution within the graft are of critical importance to maintain vascular patency and to preserve function during ischemic storage. To effectively washout the graft, preservation solutions require osmotically active and impermeable components that prevent extravasation of the preservation solution which can lead to interstitial edema and cell swelling. The solutions used in the present study all contain impermeants (Table 1). However, to enhance prevention of extravasation and tissue swelling, the UW and PS solutions incorporate colloids, as opposed to HTK. The requirements for colloid in washout solutions is controversial, because it can increase the viscosity of the solution, thereby impairing its flow through the organ. The colloid in a preservation solution is usually regarded as an inert component included solely for its oncotic properties. UW contains hydroxyethyl starch (HES), whereas PS contains polyethylene glycol (PEG). The HES molecule in UW exerts a hyperaggregating interaction with human RBCs which has been described to result in incomplete washout and stasis of blood in the graft. To date,
the use of the PEG in preservation solutions has not been associated with
disadvantages\textsuperscript{19,21}. PEG displays potent immunocamouflaging properties
whereby cells are shielded from immune recognition\textsuperscript{22,23}. PEG has been
demonstrated to inhibit the early inflammatory response associated with
ischemia-reperfusion injury and to possess antioxidative properties\textsuperscript{24,25}.
The results of the present study indicated that the presence of a colloid
facilitated an effective washout of kidneys. Moreover, the PS washout
solution combines both a colloid and a low-viscosity appearing to be the
most effective solution for kidney washout.
The amount of remaining RBCs washed out from warm ischemically
damaged kidneys was significantly lower using PS compared with HTK
solution. Because the low-viscosity HTK solution is universally considered
to be the solution of choice for the washout and preservation of kidney
grafts subjected to warm ischemia, we opted to omit UW from the WIT
30 group. Our data on WIT 0 porcine kidneys showed no clear differences
in the amount of RBCs remaining in the vascular bed after washout. This
study showed that low viscosity is not the main determinant responsible
for effective washout of organs for transplanting, because the colloid-
based PS solution provided a more effective washout than HTK in the WIT
30 groups.
The decreased weight of kidneys in the UW and PS groups after washout
could be a result of the incorporation of a colloid in both solutions. Only
kidneys washed out using HTK (WIT 0 and WIT 30) gained weight during
washout, suggesting extravasation of the preservation solution and thus
the formation of interstitial edema.
Histological evaluation of tissue specimens from warm ischemically
damaged kidneys demonstrated a greater degree of interstitial edema in
the HTK WIT 30 compared with the PS WIT 30 group. Also, specimens of
HBD kidneys taken after washout followed by 24 hours CS (WIT 0) showed
higher degrees of interstitial edema in the HTK WIT 0 group compared
with the PS WIT 0 group. Whereas the increased weight of HTK-washed
out kidneys proved to be reversible after CS, there was persistence of
the tissue injury. Overall, the structural integrity of warm ischemically
damaged kidneys was better preserved using PS for washout compared
with HTK. The morphology of kidneys washed out and cold stored without
prior warm ischemia was better preserved in the PS group compared with
either UW or HTK. The degree of interstitial edema was greater in the
UW- compared with the PS-preserved kidneys, which can be explained
by the intracellular versus extracellular electrolyte composition of UW and
PS, respectively. Extracellular-type solutions may facilitate homogeneous
perfusion of the vascular bed because they have the potential of limiting
cold-induced vasoconstriction\textsuperscript{26}. In addition, solutions with an extracellular
electrolyte composition can maintain the equilibrium between the
extracellular and intracellular compartments and thus prevent osmotic
shift and cell swelling. The findings in the present study corroborate
previous studies by our group using a porcine kidney autotransplantation
model which also demonstrated improved preservation of kidney grafts washed out and preserved using PS when compared with UW (WIT 0) and HTK (WIT 30)\textsuperscript{19,27}. A controlled clinical trial should be performed to establish the clinical significance of these findings.

**Conclusions**

This study demonstrated that washout of kidney grafts using the colloid-based preservation solutions UW and PS more rapidly decreased the core temperature compared with HTK. Also, fewer RBCs remained in the vascular bed of warm ischemically damaged kidneys after washout using PS compared with HTK. After 24 hours’ CS, overall, the structural integrity of kidneys washed out with and stored in PS was better preserved compared with those using either UW or HTK.

**Acknowledgements**

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References


