Preclinical evaluation of a new organ preservation solution
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Citation for published version (APA):

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Chapter 8

Pulsatile perfusion preservation of warm-ischaemia damaged experimental kidney grafts
Pulsatile perfusion preservation of warm-ischaemia damaged experimental kidney grafts

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Br J Surgery 2010; 97: 349–58
Abstract

Background
Cold storage using histidine-tryptophan-ketoglutarate (HTK) solution is used widely in clinical practice for the preservation of warm ischaemia-damaged kidney grafts. This study assessed the efficacy of pulsatile machine perfusion in combination with Polysol® for the preservation of warm ischaemia-damaged kidney grafts.

Methods
After induction of warm ischaemia by clamping of the left renal pedicle for 30 min, pigs were subjected to left nephrectomy. Thereafter, grafts were preserved for 20 h by cold storage with HTK (CS-HTK) or Polysol® (CS-PS), or machine preservation with Polysol® (MP-PS). Subsequently, contralateral kidneys were removed and preserved kidneys were transplanted. Control pigs underwent unilateral nephrectomy. Renal function was assessed daily for 1 week. Kidney biopsies were analysed for morphology and proliferative response.

Results
Renal function of warm ischaemia-damaged grafts preserved using MP-PS was comparable to that of non-ischaemic controls. MP-PS and CS-PS groups showed improved renal function compared with the CS-HTK group, with more favorable results for MP-PS than for CS-PS. The proliferative response of tubular cells in the CS-HTK group was higher than in all other groups.

Conclusion
This study demonstrated that the function of warm ischaemia-damaged kidney grafts after pulsatile perfusion preservation was comparable to that of non-ischaemic controls.
**Introduction**

In an era of continuous organ shortage, alternative sources such as kidneys from non-heart-beating donors are increasingly being employed for transplantation. Nevertheless, these organs have suffered from additional warm ischaemia injury and are associated with higher primary non-function and delayed graft function rates than organs from heart-beating deceased donors. Maintenance of organ viability during preservation is considered an important prerequisite for successful outcome after transplantation. This has led to renewed interest in organ preservation methods; the improvement in organ preservation methods is considered an effective means of expanding the donor pool.

Histidine-tryptophan-ketoglutarate solution (HTK) is used widely for cold storage preservation of warm ischaemia-damaged kidney grafts. However, hypothermic machine perfusion has proven to be beneficial in the preservation of warm ischaemia-damaged grafts. Recently, Moers and colleagues demonstrated the value of machine perfusion in the preservation of deceased donor kidney grafts compared with cold storage. Preclinical studies have also demonstrated the additional value of oxygenation during preservation of grafts for transplantation. However, the technology of oxygenated perfusion has not been applied in machine perfusion systems that are currently commercially available. The Lifeport® (Organ Recovery Systems, Des Plaines, Illinois, USA) and the RM3® (Waters Medical Systems, Plymouth, Minnesota, USA) do not allow active oxygenation of the perfusion medium. Recently, a disposable perfusion system for oxygenated hypothermic perfusion preservation of kidney and liver grafts has been developed, the Airdrive® (Doorzand, Airdrive, Amsterdam, The Netherlands). Polysol® (Doorzand Polysol, Amsterdam, The Netherlands), a low-viscosity perfusion solution, was developed for use with the Airdrive®. In a previous study of cold storage preservation of heart-beating porcine kidney grafts, Polysol® improved microcirculation and renal function compared with grafts preserved using University of Wisconsin solution (UW).

The aim of this study was to assess the efficacy of hypothermic pulsatile perfusion for the preservation of ischaemia-damaged kidney grafts using a porcine autotransplantation model. Cold storage preservation using HTK or Polysol® was compared with pulsatile perfusion using the Airdrive® in combination with Polysol®.

**Methods**

All experiments were performed according to the institutional guidelines of the Animal Ethics Committee of the University of Amsterdam following The Principles of Laboratory Animal Care (National Institutes of Health...
Female Landrace pigs of mean (SD) weight 28.5 (2.3) kg were allowed to acclimatize to their surroundings for a minimum of 1 week before surgery. Before experiments, the animals were fasted overnight with free access to water.

**Experimental design**

Four groups were assigned randomly: cold storage using HTK (CS-HTK; \( n=6 \)), cold storage using Polysol® (CS-PS; \( n=6 \)), machine perfusion using the Airdrive® in combination with Polysol® (MP-PS group; \( n=6 \)) and unilateral nephrectomy controls (\( n=4 \)). It was decided to limit the control group to four animals. Randomization of all groups was performed using a roulette wheel before the start of the study. Warm ischaemic damage was induced by clamping the renal vessels of the left kidney for 30 min. After left nephrectomy, kidneys were flushed ex vivo via the renal artery with 500 ml of the appropriate preservation solution at 4 °C at a hydrostatic pressure of 100 cmH₂O. As the Airdrive® uses Polysol® as perfusion medium, Airdrive®-perfused grafts were flushed with Polysol® before preservation. After flushing, kidneys were preserved for 20 h according to the protocol assigned. Kidney grafts were weighed directly after retrieval, after washout and before reimplantation. Animals in the experimental groups subsequently had renal autotransplantation as described previously.

**Anaesthetic protocol**

The anaesthetic protocol was identical for nephrectomy and autotransplantion procedures. Animals were premedicated with 10 mg/kg ketamine, 2 mg/kg azaperone and 0.02 mg/kg atropine, administered intramuscularly. General anaesthesia was induced by inhalation of a mixture of oxygen/air and isoflurane. After intubation, anaesthesia was maintained by mechanical ventilation and intravenous administration of ketamine (5-10 mg per kg bodyweight per h), sufentanil (5-10 µg per kg per h), pancuronium (50-100 µg per kg per h) and, if necessary, isoflurane (0-2 per cent). For infusion and daily collection of blood samples, the right internal jugular vein was cannulated with a polyethylene catheter that was tunneled through the skin. Animals were monitored during surgery by means of pulse oximetry using a tail probe.

**Cold static storage**

After washout, grafts for cold storage were placed in a sterile bag filled with 500 ml of either HTK (Dr Franz Köhler Chemie, Alsbach-Hänlein, Germany) or Polysol®. Subsequently, this bag was stored on melting ice in a polystyrene box for 20 h.
Machine perfusion
Immediately after flushing with Polysol®, kidneys were placed in the Airdrive® hypothermic perfusion system (Fig. 1) and perfused with 2 litres Polysol® at 2-6 °C for 20 h. This is a disposable perfusion system for hypothermic oxygenated pulsatile perfusion of kidney and liver grafts. The Airdrive® incorporates a 2-litre oxygen cylinder (more than 99 per cent oxygen) which is part of an innovative perfusion technology. The oxygen is used to propel the pump and to oxygenate the perfusion medium by means of a specially designed oxygenator. To maintain sterility, the oxygen pressure is used to create an overpressure in the organ chamber. Cold packs are used to keep the temperature of the system at less than 10 °C for at least 24 h of storage. During operation, the system records flow, temperature and resistance, and these values can be transferred via a USB connection to a Windows®-based computer. In this study a mean perfusion pressure of 20 mmHg was applied.

Renal autotransplantation
After preservation, general anaesthesia was induced and the abdomen reopened. The contralateral kidney was removed and the preserved kidney transplanted heterotopically. The renal vein was anastomosed end to side to the inferior vena cava, and the renal artery end to end to the right renal artery. Both anastomoses were performed using 6/0 polypropylene running sutures. Before completion of the arterial anastomosis, a bolus of 3000 units heparin was injected intravenously to prevent vascular thrombosis. Immediately after reperfusion, 500 ml 20 per cent glucose was administered to induce an osmotic diuresis. The ureter was cannulated with a polyethylene catheter to allow free outflow of urine through a ureterocutaneostomy. The catheter was exteriorized through a right abdominal wall incision and connected to a urine collection bag which was held in place by a custom-made vest worn by the animal to enable continuous collection of urine while allowing the animal free movement. The duration of cold ischaemia and the time taken to construct both anastomoses was recorded. Control animals had a unilateral nephrectomy followed by cannulation of the ureter of the contralateral, non-ischaemic kidney 20 h later.

Following transplantation or unilateral nephrectomy, animals were observed for 7 days. Venous blood samples were taken daily for the assessment of renal function measurement of by serum creatinine, blood urea and electrolytes. Twenty-four-hour urine production was measured and creatinine clearance (urinary creatinine × urinary volume/plasma creatinine) was calculated. Fractional excretion of sodium was calculated daily for all animals using the formula ((urinary sodium × plasma creatinine)/(plasma sodium × urinary creatinine)) × 100.

Seven days after transplantation, before the animal was killed, kidneys were retrieved under general anaesthesia for histological evaluation. All procedures were performed by the same surgical team.
Histology
Structural integrity was assessed using light microscopy. Kidney specimens were fixed with neutral 10 per cent buffered formalin and embedded in paraffin. Conventional staining with haematoxylin and eosin and periodic acid-Schiff was employed. Tissue sections of cortex, medulla and the corticomedullary junction were evaluated by a pathologist blinded to the experimental conditions. Tissue injury in each specimen was graded in ten randomly chosen, non-overlapping fields (×400 magnification). A previously described semiquantitative score was used to quantify glomerular damage, tubular injury, inflammatory infiltration, interstitial edema and necrosis12-13.
For electron microscopy, samples of 2×2×2 mm were fixed in a Karnovsky solution and prepared as described previously12. Sections were examined by a pathologist blinded to the experimental conditions using a CM10 transmission electron microscope (FEI, Philips, Eindhoven, The Netherlands). Images were acquired using a digital transmission electron microscopy camera (Morada 10-12; Soft Imaging System, Soest, The Netherlands) using the software Research Assistant.

Immunohistochemistry
For the detection of proliferating cells, immunostaining was performed on paraffin-embedded tissue by applying a rabbit polyclonal antibody against nuclear antigen Ki-67 (Abcam, Cambridge, UK) using the Dako REAL™ Detection System (peroxidase/DAB+ rabbit/mouse; Dako, Hamburg, Germany). After removal of paraffin wax and rehydration, slides were boiled in citrate buffer (pH 6) and hydrogen peroxidase blocking solution was applied. Slides were then incubated with the primary antibody (1 : 1000) for 30 min followed by Dako Wash Buffer and with biotinylated secondary antibodies.
(Dako Real Link), and developed using 1 per cent 3,3-diaminobenzidine (Dako REALTM DAB+ Chromogen) diluted in buffered solution containing hydrogen peroxide. An isotype control antibody was used to prepare a background staining control. Slides were counterstained with haematoxylin. All kidney sections were examined by an observer blinded to the groups. The number of Ki-67-positive tubular epithelial cells was quantified in ten non-overlapping fields (magnification × 400) in the corticomedullary junction 14.

**Statistical analysis**
Data are presented as mean (SD). Statistical analysis was by ANOVA followed by the Bonferroni post-test correction. Area under the curve (AUC) for serum creatinine, blood urea and creatinine clearance was calculated individually using the GraphPad Prism® 5.0 statistics package (GraphPad Software, San Diego, California, USA). $P < 0.050$ was considered significant.

**Results**
The pigs’ weights did not differ between groups ($P = 0.473$). All pigs had normal renal function before the experiments and all survived 7 days.

**Kidney weight**
Kidney weight after retrieval did not differ between the groups (CS-HTK 83.3 (11.2) g, CS-PS 81.3 (9.6) g, MP-PS 89.2 (13.7) g; $P = 0.496$). Overall, there were no differences between the groups in kidney weight before flushing, after flushing and after preservation ($P = 0.405$, repeated measures ANOVA). However, during preservation, kidneys preserved by machine perfusion with Polysol® gained 15.5 (5.9) per cent, whereas cold-stored kidneys lost weight (CS-HTK – 28.5 (2.5) per cent, CS-PS – 17.2 (4.9) per cent).

**Machine perfusion**
All grafts in the MP-PS group were perfused at a constant pressure of 20 mmHg. During perfusion, all grafts had an increase in flow rate, whereas perfusion resistance decreased over time (resistance = pressure/flow) (Fig. 2). Kidney temperature after 20 h of machine perfusion was 5.8 (0.8) °C.

**Renal autotransplantation**
Cold ischaemia times were similar between the groups (CS-HTK 20.15 (0.20) h; CS-PS 20.13 (0.42) h; MP-PS 20.22 (0.17) h; $P = 0.903$). The time taken to perform both anastomoses was comparable (CS-HTK 42.0 (9.7) min; CS-PS 46.7 (24.3) min; MP-PS 62.5 (27.5) min; $P = 0.267$).
Renal function
Apart from three of six animals in the CS-HTK group that did not produce urine during the first 24 h after reperfusion, all animals produced urine shortly after reperfusion. The serum creatinine AUC was lower in the MP-PS and CS-PS groups than in the CS-HTK group. The serum creatinine AUC of MP-PS grafts was similar to that of non-transplanted controls (Fig. 3A). Blood urea levels showed the same trend; the AUC for blood urea in the CS-HTK group was higher than that in both the CS-PS and the MP-PS group. The blood urea AUCs of the CS-PS and MP-PS groups were comparable to that in the control group (Fig. 3B). Among autotransplanted animals, peak

**Fig. 2.** Mean (SD) machine perfusion parameters during 20 h of perfusion with Polysol® using the Airdrive® (n = 6): (A) perfusion flow and (B) perfusion resistance.
values of serum creatinine were lowest in the MP-PS group, and lower in the CS-PS group than in the CS-HTK group. Peak values of blood urea were similarly lowest in the MP-PS group (Table 1).

The AUC for creatinine clearance of MP-PS grafts was higher than that for CS-HTK and CS-PS grafts, and comparable to that for controls (Fig. 3C). The AUC for fractional excretion of sodium was highest in the CS-HTK group ($P < 0.010$ versus CS-PS, $P < 0.001$ versus MP-PS and control). Values in the other three groups were similar.

### Table 1. Summary of results after transplantation

<table>
<thead>
<tr>
<th></th>
<th>CS-HTK</th>
<th>CS-PS</th>
<th>MP-PS</th>
<th>Control</th>
<th>$P$-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day survival</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>4/4</td>
<td>-</td>
</tr>
<tr>
<td>Peak creatinine</td>
<td>1016 ± 97.1†</td>
<td>629.8 ± 158.8‡</td>
<td>286.7 ± 90.6</td>
<td>170.8 ± 54.2</td>
<td>&lt;0.001</td>
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<tr>
<td>(µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$T_{peak}$ creatinine</td>
<td>3.2 ± 0.8</td>
<td>1.8 ± 0.4</td>
<td>1.5 ± 1.1</td>
<td>2.3 ± 2.1</td>
<td>0.088</td>
</tr>
<tr>
<td>(days)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>297.5 ± 210.4</td>
<td>167.3 ± 45.1</td>
<td>120.2 ± 7.7</td>
<td>115.0 ± 13.8</td>
<td>0.050</td>
</tr>
<tr>
<td>at time of death</td>
<td></td>
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<tr>
<td>(µmol/L)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Peak blood urea</td>
<td>23.8 ± 5.2‡</td>
<td>16.5 ± 5.3§</td>
<td>10.0 ± 3.4</td>
<td>6.5 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{peak}$ urea</td>
<td>3.0 ± 0.6</td>
<td>1.5 ± 0.8</td>
<td>1.7 ± 1.2</td>
<td>2.8 ± 2.8</td>
<td>0.159</td>
</tr>
<tr>
<td>(days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood urea at</td>
<td>7.6 ± 6.6</td>
<td>3.0 ± 1.0</td>
<td>2.3 ± 0.9</td>
<td>2.1 ± 0.4</td>
<td>0.060</td>
</tr>
<tr>
<td>time of death</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are mean ± SD. Animals were killed on day 7. CS, cold storage; HTK, histidine-tryptophane-ketoglutarate solution; PS, Polysol®, $T_{peak}$, time to peak level; *One-way ANOVA followed by Bonferroni’s multiple comparison test. †$P < 0.001$ versus CS-PS, MP-PS and control; ‡$P < 0.001$ versus MP-PS and control; §$P < 0.050$ versus control (Bonferroni’s multiple comparison test).
Fig. 3. Mean (SD) (A) serum creatinine levels, (B) blood urea levels and (C) creatinine clearance rates after transplantation of grafts following cold storage with histidine-tryptophan-ketoglutarate solution (CS-HTK group), cold storage with Polysol® (CS-PS group) or machine perfusion with Polysol® (MP-PS group), and in controls that underwent unilateral nephrectomy. (A) \( P < 0.050 \), CS-HTK versus CS-PS, MP-PS and control; \( P < 0.050 \), CS-PS versus MP-PS and control. (B) \( P < 0.050 \), CS-HTK versus CS-PS, MP-PS and control. (C) \( P < 0.050 \), CS-HTK versus MP-PS and control; \( P < 0.050 \), CS-PS versus MP-PS and control (comparison of areas under the curve; one-way ANOVA followed by Bonferroni’s multiple comparison test).
Histology
Histological evaluation of the warm ischaemia-damaged grafts showed less glomerular damage in the CS-PS group than in the CS-HTK group. The degree of tubular damage was less in the MP-PS grafts than in grafts subjected to cold storage with either HTK or Polysol®. The same trend was seen for inflammatory infiltration in the tissue, as well as for the presence of interstitial edema. Necrosis was most commonly seen in grafts kept in cold storage with HTK. Except for glomerular shrinkage, tissue injury of warm ischaemia-damaged grafts preserved using MP-PS was comparable with that of non-transplanted controls (Table 2).

Table 2. Quantification of morphological data at time of death

<table>
<thead>
<tr>
<th></th>
<th>CS-HTK</th>
<th>CS-PS</th>
<th>MP-PS</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
</table>
| **Glomerular damage**    | 1.4 ± 0.8 | 0.7 ± 0.8 | 1.1 ± 0.8 | 0.4 ± 0.4 | CS HTK vs. CS PS *  
CS HTK vs. Control ***  
MP PS vs. Control * |
| **Tubular damage**       | 3.1 ± 0.4 | 2.4 ± 0.5 | 1.7 ± 0.3 | 1.5 ± 0.6 | CS HTK vs. CS PS ***  
CS HTK vs. MP PS ***  
CS HTK vs. Control ***  
CS PS vs. MP PS ***  
CS PS vs. Control *** |
| **Inflammatory infiltration** | 1.9 ± 0.4 | 1.0 ± 0.5 | 0.4 ± 0.4 | 0.4 ± 0.4 | CS HTK vs. CS PS ***  
CS HTK vs. MP PS ***  
CS HTK vs. Control ***  
CS PS vs. MP PS ***  
CS PS vs. Control *** |
| **Interstitial edema**   | 2.1 ± 0.9 | 1.0 ± 0.9 | 0.4 ± 0.5 | 0.4 ± 0.6 | CS HTK vs. CS PS ***  
CS HTK vs. MP PS ***  
CS HTK vs. Control ***  
CS PS vs. MP PS *  
CS PS vs. Control * |
| **Necrosis**             | 0.7 ± 0.4 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 | CS HTK vs. CS PS ***  
CS HTK vs. MP PS ***  
CS HTK vs. Control *** |

Values are mean ± SD. Semiquantitative scale: 0 = no abnormality; 1, mild lesions affecting ≤ 10% of the field; 2, 10%-25%; 3, 25%-50%; 4, 50%-75%; 5, extensive damage with involvement of > 75% of the field; CS, cold storage; HTK, histidine-tryptophane-ketoglutarate solution; PS, Polysol®; MP, machine perfusion. *P<0.050, ***P<0.001.
Electron microscopic evaluation showed well preserved structural integrity of warm ischaemia-damaged kidney grafts stored using MP-PS and CS-PS, comparable with that in kidneys in the control group. Tubular epithelial cells (and glomerular capillary endothelium) were well preserved, whereas grafts in the CS-HTK group showed pyknotic nuclei, vacuolization of tubular cells and few mitochondria. Mitochondrial structures were better preserved in the MP-PS group than in the CS-HTK group (Fig. 4).

**Fig. 4.** Electron microscopy. (A) Control (unilateral nephrectomy) group; normal tubular epithelium, and intact nuclei (N) and mitochondria (M). (B) Cold storage with histidine-tryptophan-ketoglutarate solution (CS-HTK group); tubular epithelium shows pyknotic nuclei, major vacuolization of the cytoplasm (V) and few mitochondria. (C) Cold storage with Polysol® (CS-PS group); tubular epithelium is well preserved. Nuclei and mitochondria are intact. Debris (D). (D) Machine perfusion with Polysol® (MP-PS group); nuclei and mitochondria are well preserved. TBM, tubular basal membrane; TL, tubular lumen (original magnification × 4000).
Immunohistochemistry

Immunostaining for the detection of proliferating cells showed more proliferating tubular epithelial cells in the CS-HTK group than in all other groups. No differences were seen between the CS-PS, MP-PS and control groups (Fig. 5).

Discussion

This study has shown that full functional recovery of warm ischaemia-damaged kidney grafts can be achieved by hypothermic pulsatile machine perfusion using the Airdrive® system. The structural integrity of predamaged grafts preserved using the system was comparable to that of normal non-transplanted controls. In addition, renal function of grafts subjected to 30 min of warm ischaemia followed by 20 h of cold ischaemia was better with MP-PS than with cold storage using Polysol® or HTK. For the cold-stored grafts, preservation using Polysol® produced more favourable results than preservation with HTK. Nicholson and colleagues also compared cold storage and machine perfusion preservation of predamaged kidney grafts using a porcine autotransplant model. Their studies, using UW for cold storage or Belzer machine preservation solution (MPS) in combination with the Waters RM3® machine perfusion system for the preservation of warm ischaemia-damaged grafts, did not establish any advantage of machine perfusion over cold storage. Their warm ischaemia time was equivalent (30 min) but their cold ischaemia time (24 h) was slightly longer than in the present
study. Based on the poor survival results (two of five animals survived 7 days, whereas one of five animals survived 14 days, in both cold storage and machine perfusion groups), the chosen conditions of warm and cold ischaemia may have been an excessive insult. In addition, it was suggested that the porcine kidney was more sensitive to 24 h cold preservation than the canine kidney, which has been preserved successfully for 72 h\textsuperscript{16}. A study by Minor and co-workers\textsuperscript{8} on machine perfusion of warm ischaemia-damaged porcine kidney grafts did, however, show an advantage of over cold storage. Renal function of grafts subjected to warm ischaemia for 40 min followed by cold ischaemia for 18 h was improved after oxygenated machine perfusion preservation using either Belzer MPS or low-flow machine perfusion using HTK compared with cold storage preservation using HTK. Serum creatinine levels rose after transplantation but remained stable until the day of death 7 days later. Although machine perfusion with both Belzer MPS and HTK showed favourable results compared with cold storage using HTK, full functional recovery of grafts could not be established.

The present study did not include a MP-HTK group because HTK is considered not applicable for perfusion at pressures and flow rates inherent to the Airdrive\textsuperscript{®} system. The lack of colloids and impermeants in HTK, which are required to prevent extravasation of the perfusion medium, would inevitably result in tissue swelling.

The benefit of oxygenated machine perfusion over cold storage preservation is in accordance with results from other studies evaluating the addition of oxygen to the perfusate during preservation\textsuperscript{9,10,17,18}. Maathuis and colleagues\textsuperscript{17} also demonstrated the beneficial effect of active oxygenation on tubular cells as attested by reduced formation of reactive oxygen species, especially when using low arterial pressure perfusion. In the present study, the degree of tubular damage was least in the MP-PS grafts. Moreover, except for glomerular shrinkage, tissue injury of the grafts preserved using machine perfusion with Polysol\textsuperscript{®} was comparable to that of controls not subjected to either warm or cold ischaemic damage. This was associated with higher creatinine clearance rates in the MP-PS group.

In line with previous studies of cold preservation using Polysol\textsuperscript{® 12,19,20}, the present findings demonstrated the benefit of this colloid-based low-viscosity solution compared with HTK. In the clinical setting, HTK is considered the preservation solution of choice for non-heart-beating donor grafts. The low viscosity of HTK leads to a better washout of red blood cells than the high-viscosity UW\textsuperscript{21}. The combination of low viscosity and inclusion of impermeants as well as a colloid in Polysol\textsuperscript{®} possibly account for the improved preservation quality over HTK. Moreover, Polysol\textsuperscript{®} appears to meet the requirements for machine perfusion preservation, as the low viscosity prevents extravasation of perfusate resulting in tissue oedema, and attenuation of flow is prevented by the colloid and impermeants. Potential explanations for the higher efficacy of Polysol\textsuperscript{®} over UW, albeit...
in the setting of cold storage, have been described previously\textsuperscript{12}. In the present study, washout and preservation using Polysol\textsuperscript{®} yielded superior grafts to washout and cold storage with HTK. Urine production occurred directly after reperfusion in both CS-PS and MP-PS grafts, whereas three of six in the CS-HTK group did not produce urine within the first 24 h. Overall, renal function was improved and tissue integrity was preserved in both CS-PS and MP-PS groups. Evidence for improved preservation of the renal tubules in the Polysol\textsuperscript{®}-preserved groups can be derived from lower levels of fractional excretion of sodium and significantly less expression of the Ki-67 antigen than in the CS-HTK group. Expression of the Ki-67 antigen is low in normal tubular epithelium but upregulated in injured renal tubular epithelial cells\textsuperscript{22-23}.

The Airdrive\textsuperscript{®} system is pressure controlled and generates pulsatile flow. In this study, the mean pressure preset was 20 mmHg, which is a lower than proposed by Maathuis and colleagues\textsuperscript{17}. In a study using the Groningen hypothermic machine perfusion system (Kidney Assist\textsuperscript{®}; Organ Assist, Groningen, The Netherlands), UW cold storage preservation was compared with machine perfusion preservation using a pressure of 60/40 or 30/20 mmHg. High-pressure perfusion resulted in poor survival and endothelial damage due to higher shear rates\textsuperscript{17}. Previous experiments showed that a pressure of 30 mmHg applied in the Airdrive\textsuperscript{®} system was not favourable for the perfusion of porcine kidneys (M.C. Schreinemachers and B.M. Doorschot, unpublished data). Yland and co-workers\textsuperscript{24} concluded that pressure-controlled perfusion is advantageous as the perfusate flow is adjusted automatically to the vascular resistance or size of the organ, thereby facilitating safe perfusion of organs of any size. The application of a lower perfusion pressure preset in the Airdrive\textsuperscript{®} possibly contributed to its beneficial effects, especially in these compromised grafts. Continuous perfusion leads to prolonged washout of blood remnants and waste products while the oxygenated perfusate is distributed evenly, without the detrimental effects to the endothelium that occur during high-pressure perfusion. The rise in perfusion flow and the decrease in perfusion resistance observed in the present study supported this hypothesis and corroborated the suggestion that an increase in perfusion flow at a preset perfusion pressure could be a reliable indicator of graft viability in other studies\textsuperscript{25,26}.

Another possible indicator of viability is the increase in weight during machine perfusion\textsuperscript{27}. An increase in graft weight during machine perfusion is associated with extravasation of perfusate, resulting in tissue oedema, and is considered to be a negative parameter of graft viability\textsuperscript{24,27,28}. However, the prognostic value of weight increase was not supported in the present study as tissue oedema was significantly less in the MP-PS group than in both cold storage groups after 7 days. The degree of interstitial oedema in MP-PS grafts was comparable to that of non-ischaemic controls. The finding that weight increase had no prognostic significance may be explained by the fact that weight increase of grafts was limited to 15 per
cent, as seen in the low-flow perfusion group described by Yland and colleagues\textsuperscript{24}. Low-flow perfusion in their study compared favourably with high-flow perfusion\textsuperscript{24}.

The Airdrive\textsuperscript{®} system used here has been designed for hypothermic machine perfusion with a back-up of cold storage in case of mechanical failure. The system is capable of preserving kidneys as well as livers, but it remains portable (weighing 8 kg overall). The high costs and complex logistics associated with current machine perfusion systems are circumvented by the disposable nature of the system as return transport, maintenance and dedicated personnel involved in operation and handling are no longer needed.

In this study the Airdrive\textsuperscript{®} system proved a reliable and highly effective method of organ preservation, resulting in kidney function comparable to that of normal non-transplanted kidneys. However, there were several limitations to the present study. As tissue sampling was limited to the seventh day after transplantation, events directly after reperfusion were not investigated. Furthermore, the question of whether the favourable results were due to active oxygenation or the low-pressure pulsatile perfusion remains unanswered.

**Acknowledgements**

Marie-Claire Schreinemachers and Benedict Doorschodt contributed equally to this work. The authors thank Goos Huijzer, Albert van Wijk, Bob van Raalte, Mareike Schulz and Roya Soltan for their technical contributions.
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