Citation: Clin Transl Sci (2021) 14, 544–549; doi:10.1111/cts.12906

ARTICLE

Reduction of Renal Preservation/Reperfusion Injury by Controlled Hyperthermia During *Ex Vivo* Machine Perfusion

Thomas Minor[1,*,†] and Charlotte von Horn[1,†]

The possible reno-protective effect of a controlled brief heat-shock treatment during isolated *ex vivo* machine perfusion of donor grafts prior to reperfusion should be investigated in a primary *in vitro* study. Porcine kidneys ($n = 14$) were retrieved after 20 minutes of cardiac standstill of the donor and subjected to 20 hours of static cold storage in University of Wisconsin solution. Prior to reperfusion, kidneys were subjected to 2 hours of reconditioning machine perfusion with gradual increase in perfusion temperature up to 35°C. In half of the kidneys ($n = 7$), a brief hyperthermic impulse (10 minutes perfusion at 42°C) was implemented in the machine perfusion period. Functional recovery of the grafts was observed upon normothermic reperfusion *in vitro*. Hyperthermic treatment resulted in a 50% increase of heat shock protein (HSP) 70 and HSP 27 mRNA and was accompanied by ~ 50% improvement of tubular re-absorption of sodium and glucose upon reperfusion, compared with the controls. Furthermore, renal loss of aspartate aminotransferase was significantly reduced to one-third of the controls as was urinary protein loss, evaluated by the albumin to creatinine ratio. It is concluded that *ex vivo* heat-shock treatment seems to be an easily implementable and promising option to enhance renal self-defense machinery against reperfusion injury after preservation that merits further investigation in preclinical models.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The induction of heat-shock proteins (HSPs) in local tissues is a well-known defense mechanism in response to environmental stresses. Upregulation of those molecular chaperones induces repair mechanisms and tolerance against subsequent cellular injury. Clinical kidney transplantation more and more relies on the use of extended criteria donor organs that bear an enhanced susceptibility to ischemia/reperfusion injury. *Ex vivo* machine perfusion of marginal organ grafts provides a privileged access to the graft and a new perspective for an easy and controlled application of a hyperthermic stimulation to the isolated organ in order to trigger heat shock responses and unleash signal pathways that favorably affect consecutive exacerbation of tissue injury and improve renal recovery after transplantation.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ We examine a potential protective effect of a brief hyperthermic stimulus during *ex vivo* pre-transplant kidney perfusion on functional outcome upon postischemic reperfusion.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study gives some indications that hyperthermic treatment during *ex vivo* renal machine perfusion acts as a possible novel tool for the improvement of functional outcome of marginally preserved kidneys upon postischemic reperfusion. Data support a relevant role of HSPs in reduction of cellular destruction and improvement of posttransplantation tubular kidney function.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The addition of brief controlled hyperthermia to an established normothermic machine perfusion protocol is an easily implementable method for clinical application and may contribute to improved organ recovery after transplantation.

Clinical kidney transplantation more and more relies on the use of extended criteria donor organs that have been predamaged prior to retrieval or are likely less than optimal quality. Along with the increasing proportion of those organs that bear an enhanced susceptibility to injurious triggers upon ischemic preservation,[1,2] tissue alterations

[†]Both authors contributed equally to the study.

[1]Surgical Research Department, Clinic for General, Visceral and Transplantation Surgery, University Hospital Essen, University Duisburg-Essen, Essen, Germany. *Correspondence: Thomas Minor (chirfor@uk-essen.de)

Received: June 10, 2020; accepted: September 3, 2020. doi:10.1111/cts.12906

related to ischemia/reperfusion injury do represent a growing major issue in renal transplantation that triggers cellular dysfunction upon reperfusion.

The induction of cellular chaperones and triggering of a protective signal phenotype (e.g., by subcritical thermal stress), is a well-known mechanism to reduce tissue alterations pertinent to a later stress situation and challenge to tissue homeostasis.[3,4]

The advent of isolated machine perfusion as alternative or adjunct to ischemic cold storage of marginal donor grafts has opened a new perspective for an easy and controlled application of a hyperthermic challenge to the isolated organ in order to trigger a heat-shock response and unleash signal pathways that favorably affect consecutive exacerbation of tissue injury and improve renal recovery after transplantation.

The preconditioning effect of the heat-shock response has been evidenced experimentally to be related to a temporary supra-normal increase of cytoresistance[5] as well as being operative via enhancement of secondary cellular repair mechanisms.[6] More recently it has been shown that the induction of heat-shock proteins (HSPs) may confer protection even when induced early after the original insult to the tissue has already taken place.[7]

The privileged access to the isolated organ precludes cumbersome requirements as for whole body heating and incurs adverse side effects on donor or recipient organisms.

Therefore, this study was intended as a pilot investigation to scrutinize for primary evidences of hyperthermic treatment during *ex vivo* renal machine perfusion to act as a possible novel tool for the improvement of functional outcome of marginally preserved kidneys upon postischemic reperfusion.

METHODS

All experiments were carried out on kidneys that were retrieved from dead female German Landrace pigs (age: 10–12 weeks, weight: 25 and 30 kg). Federal law regarding the protection of animals and principles of laboratory animal care (National Institutes of Health (NIH) publication no. 85-23, revised 1985) were followed. Animals were treated according to the rules and after approval of the local authorities.

Kidneys were removed 20 minutes after circulatory standstill and flushed on the back-table by 100 cm gravity with cold (4°C) University of Wisconsin solution until the effluent became clear. No heparin was given at any time.

Grafts were subsequently stored for 18 hours in a beaker filled with University of Wisconsin solution and a temperature regulated to 4°C by means of a cryothermostat.

In the control group, kidneys were then subjected to 2 hours of oxygenated (95% O_2; 5% CO_2) normothermic machine perfusion with Aqix RS I solution supplemented with 40 g/L of bovine serum albumin. As proposed earlier,[8,9] perfusion was started in hypothermia followed by a graduated increase up to 35°C within the first 60 minutes, controlled by a programmable circulating cryothermostat.

In the experimental group, a brief period of controlled hyperthermia (CH) was included in the isolated perfusion protocol. After reaching 35°, the temperature of the perfusate was temporarily elevated further to 43°C for 10 minutes (**Figure 1**) prior to continuation of the perfusion at 35°C for the rest of the 2-hour period. Time and height of the temperature increase have been derived from preceding incremental screening experiments aiming to obtain a consistent effect.

In three experiments, oxygen partial pressure in the tissue was controlled by the use of a microfiber needle type optic oxygen sensor, connected to a temperature compensated fiber optic oxygen meter (Microx 4, PreSens precision sensing, Regensburg, Germany), which simultaneously recorded the corresponding temperature by means of a thermistor probe.

The needle was placed at the inner cortical region of the kidney in order to document adequate tissue oxygenation even during hyperthermic periods of perfusion.

Isolated kidney reperfusion model

Renal recovery from preservation/reperfusion injury was evaluated using an established *in vitro* reperfusion model, as described previously.[10]

In brief, kidneys were cold flushed with 100 mL of saline solution at the end of the machine reconditioning period and then kept at room temperature for 20 minutes as to simulate the second warm ischemia period during surgical implantation *in vivo*. This time was also used for cannulation of the ureter with a large tubing to allow for urine collection upon reperfusion.

Kidneys were placed in a thermostatically controlled plexiglass chamber and perfused via the renal artery at a mean pulsatile pressure of 90 mmHg. The venous effluent drained free into the reservoir. Oxygenation of the perfusate was performed with 95% O_2/5% CO_2 using a thermo-controlled hollow fiber oxygenator. Arterial pressure was maintained by means of a servo-controlled roller pump in conjunction with a pressure transducer connected to the arterial perfusion cannula. Urine was collected from the PE-tubing inserted into the ureter that was guided to the outside of the reservoir. Every 100 mL of collected urine were filtered and re-infused to the reservoir in order

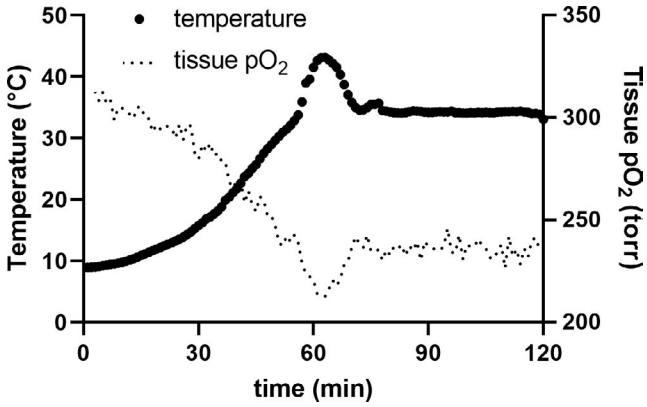

Figure 1 Renal cortical tissue pO_2 (dotted line) during *ex vivo* machine perfusion in relation to the respective perfusate temperature (solid line).

to maintain constant electrolyte composition of the perfusate over time.

Analytical procedures

Oxygen partial pressure and perfusate concentrations of glucose and electrolytes were measured in a pH-blood gas analyzer (ABL 815flex acid-base laboratory, Radiometer, Copenhagen).

Enzyme activities of aspartate aminotransferase (AST) and concentrations of creatinine were determined in a routine fashion by reflectance photometry on a Reflotron Plus point of care unit (Roche Diagnostics, Mannheim, Germany).

Renal clearance was calculated for the respective intervals as urinary creatinine × urine flow/perfusate creatinine.

The albumin concentration in the urine fraction at the end of the experiment was measured photometrically using the dye-binding assay with bromocresol green at the laboratory center of the university hospital and the amount of protein was normalized against the corresponding concentrations of creatinine as urinary albumin to creatinine ratio.

The filtered load of sodium (FL Na) was calculated as $FL = GFR \times Na_{(perfusate)}$.

Fractional re-absorption of sodium (FR Na) has been determined according to:

$$FRNa = (FLNa - Na_{(urine)} \times urineflow)/FLNa$$

Filtered load and fractional reabsorption of glucose were calculated accordingly.

Reverse transcription and subsequent polymerase chain reaction

Total RNA was isolated from snap frozen tissue samples from the outer cortex and analyzed as described previously.[8]

Baseline values were calculated from tissue samples that had been taken from vital kidneys *ex vivo*. Results were quantified using the $\Delta\Delta C_t$ method. Target genes were normalized to glyceraldehyde 3-phosphate dehydrogenase as internal standard. Fold gene expression values were calculated for each individual sample as normalized against baseline values.

The following primers (purchased from Qiagen GmbH, Hilden, Germany) were used in the study: glyceraldehyde 3-phosphate dehydrogenase (no. PPS00192A), HSP72 (no. PPS00074A), HSP27 (no. PPS01262), and HO-1 (no. PPS01191A).

Western Blot

Protein immunoblotting was performed from whole-cell lysates from the outer renal cortex by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretic transfer onto nitrocellulose membrane, as described previously.[8] Staining was done with Ponceau S verified uniformity of protein loading and transfer.

Incubation with primary antibodies was performed overnight at 4°C (rabbit anti-phospho extracellular signal regulated kinase (ERK, Thermo Fisher, #MA5-15174—1:500) or anti HSP 72 (StressMarq Bioscience, #SCM-100—1:300). After incubation with horseradish peroxidase-coupled secondary antibody (1:1,000) for 1 hour at room temperature, protein was detected by enhanced chemiluminescence using a C-Digit Blot Scanner (Li-COR).

Quantification of protein content was performed densitometrically with UN-SCAN-IT gel version 6.1 (Silk Scientific, Orem UT) as normalized against the expression of total ERK (Thermo Fisher, #MA5-15174) or ß-actin (Thermo Fisher, #PA1-46296).

Statistics

All values are expressed as means ±SD for $n = 7$ animals per group and differences between groups were tested by unpaired, two-sided *t*-test using Welch's correction for unequal variances, if not otherwise indicated. Statistical significance was set at $P < 0.05$.

RESULTS

Machine perfusion

During controlled oxygenated rewarming of the machine perfused grafts, temporary hyperthermia led to a visible transient drop in renal cortical tissue pO_2 (**Figure 1**), which nonetheless always remained above 200 mmHg and thus clearly complied with the physiological requirements during normothermic perfusion that had been reported to be 30–50 mmHg.[11,12]

Renal release of AST into the circulating perfusate was even slightly lower in the hyperthermia group than under control conditions (19.8 ± 12.1 vs. 33.3 ± 25.9 U/L; CH vs. control, not significant).

Molecular readout of transient hyperthermia during *ex vivo* machine perfusion

Preservation and reperfusion lead to a notable induction of HSPs even under control conditions with ~ 2-fold, 8-fold, and even 100-fold increase of mRNA transcripts of hemoxygenase 1, HSP 27, and HSP 72, respectively.

Nonetheless, controlled transient hyperthermia during machine perfusion effectively enhanced this increase in gene induction by about 50% in all cases. The effect was found to be highly significant for HSP 27 as well as HSP 72, whereas not statistical significance could be evidenced concerning the induction of hemoxygenase 1 (**Figure 2**).

Phosphorylation of ERK represents a fast, responsive protective signal event that may also promote the expression of HSP 72.[13]

It was investigated as early repercussion of heat stress on the protein level. Western blot analyses revealed that transient hyperthermia during machine perfusion significantly enhanced the amount of ERK phosphorylation in the tissue when compared with the control group (**Figure 3**). Correspondingly, protein expression of HSP 72 was also found to be significantly elevated above control values after controlled hyperthermia during machine perfusion.

Transient hyperthermia improves renal recovery after preservation

Perfusate flow during reperfusion averaged between 300 and 400 mL/minute with no differences between the two

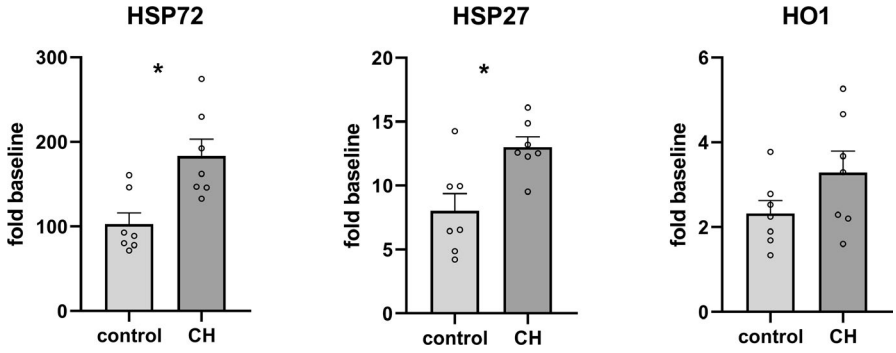

Figure 2 Transcripts of three heat-shock response genes (heat-shock protein 72 (HSP 72), HSP 27, and hemoxYgenase 1) after reperfusion of kidneys with (controlled hyperthermia (CH)) or without (control) exposure to CH during machine perfusion. Single values, mean and SEM are shown; *$P < 0.05$.

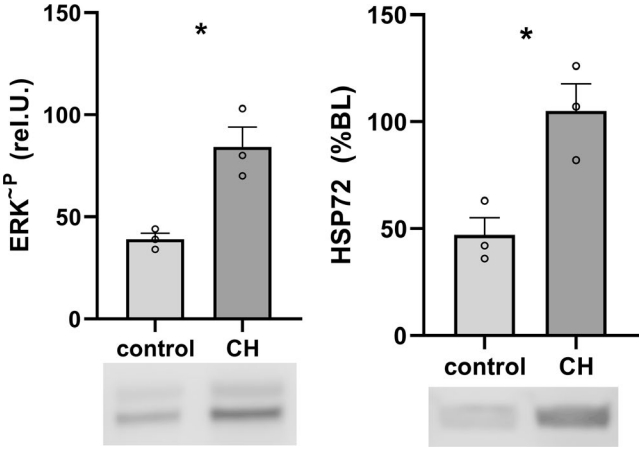

Figure 3 Left: extracellular signal regulated kinase (ERK)-phosphorylation upon reperfusion after reperfusion of kidneys with (controlled hyperthermia (CH)) or without (control) exposure to controlled hyperthermia during machine perfusion. Single values ($n = 3$), mean and SEM are shown; * $P < 0.05$. Right: Protein expression of HSP 72 after reperfusion of kidneys with (CH) or without (control) exposure to controlled hyperthermia during machine perfusion.

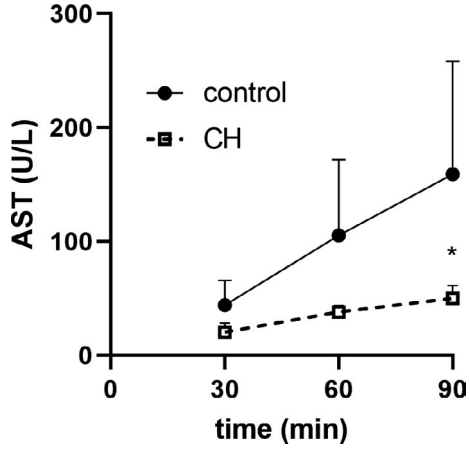

Figure 4 Enzyme release of aspartate aminotransferase (AST) upon reperfusion of kidneys with (controlled hyperthermia (CH)) or without (control) exposure to controlled hyperthermia during preceding machine perfusion (*$P < 0.05$).

groups. Urine production was also similar in both groups with 897 ± 354 vs. 601 ± 280 mL/90 minutes (control vs. CH).

Renal release of AST was taken as parameter of global cellular injury and followed during reperfusion (**Figure 4**). It is seen that hyperthermic treatment resulted in initially lower values at the onset of reperfusion and further mitigated the progressive rise during ongoing perfusion.

Renal tubular cell function was evaluated by the analysis of sodium and glucose re-absorption from the ultra-filtrate (**Figure 5**). It was found that fractional re-absorption rates from kidneys, previously subjected to controlled hyperthermia during machine perfusion, significantly surpassed those of the control group by ~ 40%.

In order to exclude the possibility that control kidneys might have been charged with absolute higher loads of sodium or glucose in the ultra-filtrate, we also determined the total filtration load of both substances, which, however, did not differ between the two groups with FL Na amounting to 2.7 ± 0.7 and 2.5 ± 0.8 mmol/minute and FL glu to 24.8 ± 12.1 and 28.6 ± 9.4 mg/min (control vs. CH, respectively).

Glomerular filtration rates were also equivocal in both groups; clearance values for creatinine amounted to 0.25 ± 0.06 mL/g/minute in the control group, and to 0.24 ± 0.08 mL/g/minute after CH.

By contrast, urinary protein leakage was significantly reduced in the kidneys that were subjected to CH (**Figure 6**).

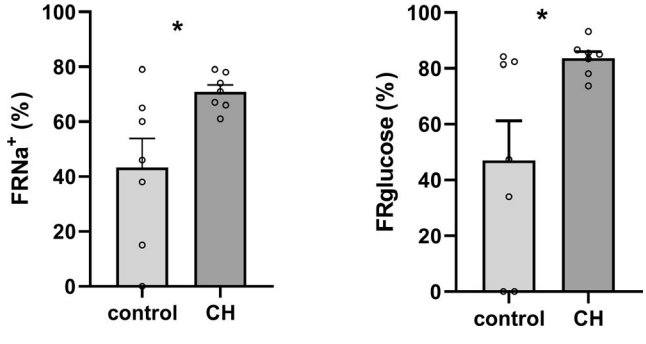

Figure 5 Renal re-absorptive capacity upon reperfusion: Fractional tubular reabsorption of sodium (FR Na, left) and fractional reabsorption of glucose (FR glu, right) of kidneys, reperfused after reconditioning. Single values, mean and SEM are shown; *$P < 0.05$. CH, controlled hyperthermia.

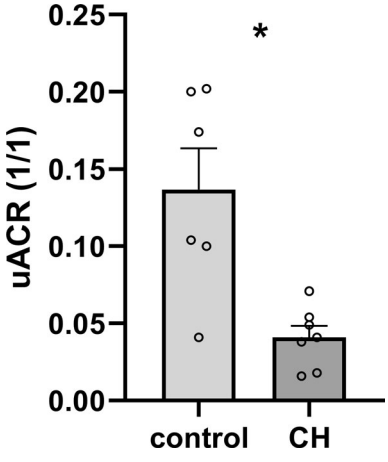

Figure 6 Renal proteinuria shown as urine albumin to creatinine ratio (UACR) of kidneys with (controlled hyperthermia (CH)) or without (control) exposure to CH.

DISCUSSION

The use of *ex vivo* machine perfusion represents an evolving trend in the management of graft preservation prior to organ transplantation.

In the last decade, hypothermic machine perfusion has been established as a superior method of preservation as compared with simple static cold storage[14,15] and became popular for the preservation of higher risk as well as extended criteria organs.[14–16]

Pulsatile stimulation of the vasculature during the extracorporeal period[17] as well as the presence of oxygen[18] were shown to maintain renal homeostasis and improve functional outcome after transplantation.

Recent technical advances have fostered a new trend toward normothermic machine perfusion as a more physiologic alternative to the hypothermic perfusion technologies, which compensates for the higher expenditure by an improved maintenance of metabolic homeostasis and a more authentic opportunity to evaluate the preserved graft prior to transplantation.[19,20]

Beyond this, normothermic machine perfusion represents an ideal platform for therapeutic interventions to the isolated organ that may serve conditioning purposes aiming to alleviate consequences of preceding retrieval/preservation injury or to precondition against later reperfusion injury after second warm ischemia and reperfusion upon transplantation.

Heat-shock treatment as a therapeutic approach was found to promote very effective protection mechanisms in the kidneys and induce tolerance to specific insults by stabilizing protein structure and cellular integrity. This effect is mainly based on the synthesis of specific HSPs, which are constitutively expressed in the kidneys and can be highly upregulated in response to a variety of stress conditions.[4]

This study shows that a brief hyperthermic challenge during isolated kidney perfusion effectively elicits a molecular heat shock reaction, as evidenced on the pre-transcriptional and on the protein level and activates survival signaling via the ERK pathway. The procedure described was thereby not associated with apparent tissue hypoxia, despite isolated perfusion without additional oxygen carriers.

It has previously been established, that the induction of HSPs may start within < 1 hour[21] and that the expression of HSP 72 increases to multitudes already after 3 hours.[22] HSP 70 is the most prominent chaperone responsive to hyperthermic stress and plays a pivotal role in tissue protection by augmentation of the cellular self-defense machinery.[5]

HSP 72 prevents misfolding of proteins,[5] facilitates the removal of damaged proteins,[6] and takes part in protein refolding and repair. In supporting cellular repair processes that are much less expensive than *de novo* synthesis. HSP 72 thus reduces metabolic needs during the critical situation upon reperfusion after cellular injury[21] and thereby helps cellular recovery from insult.

Stabilization of lysosomal membranes[23] reduces cellular injury by acid hydrolases and consecutive induction of cell death. On the molecular level, earlier studies indicate that HSP 72 activates the MEK/Erk pathway and relevant protective effect is related to Raf, MEK, and Erk phosphorylation and cell survival.[24,25] Accordingly, in our model, CH led to a significant reduction of overall release of AST, which was taken as the parameter of general cellular damage. Moreover, the progressive rise in AST during ongoing reperfusion was also notably attenuated by the treatment.

Along with this went a significant mitigation of renal dysfunction as tubular cell re-absorptive capacity was found to be increased by ~ 50% over control values.

HSP 27 is a low molecular weight HSP that was also found to be an important player in the protective heat-shock response in the kidneys.[4] Studies on rat tubular cells show that accumulation of HSP 27 in proximal tubule cells is associated with increased survival and regeneration.[26]

Based on the results of the present pilot study, the implementation of a brief hyperthermic challenge into the *ex vivo* machine perfusion protocol seems to be an easy way to condition marginally preserved kidney grafts that promotes resilience to reperfusion injury by enhancement of the cellular self-defense machinery. A controversy still exists if machine perfusion ought to be done up-front, immediately after graft procurement[27] despite of incurring logistic inconveniences, or if a brief, end-ischemic perfusion period after transport of the organ by static cold storage would be sufficient and more easily implementable in clinical routine.[28,29] *Ex vivo* conditioning of the graft by CH would be possible in either modality.

However, caution has to be taken with regard to premature final conclusions. The isolated perfused kidney (IPK) is a useful tool for functional screening studies[30] and correlations between IPK data and clinical outcomes make the IPK model a potentially useful tool for evaluation of renal function.[17,31] However, the fairly constant functional capacity of the model is limited to a few hours,[32] which precludes long-term observations.

As maximal expression of HSPs may still occur beyond this time,[22] a potential further increase of the protective effect of the hyperthermic treatment (e.g., in terms of improved regeneration after ischemic insult) will not be accounted for in our pilot setup. Moreover, it is well known from earlier studies in other models, that heat-shock treatment or lentiviral induced overexpression of HSP 27 can result in a mitigation of inflammatory re-activity of injured target organs

along with a significant reduction of neutrophil infiltration into the tissue.[33,34]

Leucocyte infiltration, in turn, is known to be a leading acute type phenomenon that triggers injurious inflammatory reactions after renal ischemia and reperfusion *in vivo*.

However, in order to exclude concomitant adverse effects of blood cell activation on artificial tubing surfaces, which would be inevitable in an extended *in vitro* model, actual transplantation and reperfusion under physiologic conditions, including natural vessel morphology, appears to be mandatory to accurately address cellular inflammatory effects, which were therefore not addressed in the present pilot investigation.

At last, recent findings demonstrated substantial sex differences in transcripts expressed in proximal tubule cells of males vs. females that may reflect sex-related disparities in chronic kidney disease or pro-inflammatory gene modules.[35] In our study, only female animals were used, thus circumventing putative sex related disparities in the heat-shock reactions. Possibly different behavior of male tissue, on the other hand, has not been addressed and can thus not be accounted for in this study.

In conclusion, our data so far show that brief hyperthermic *ex vivo* perfusion is an easy way to chaperone induction in the isolated renal graft that confers parenchymal protection upon early reperfusion and warrants further confirmation using *in vivo* transplantation models with extended observation times.

Acknowledgments. The authors gratefully acknowledge the valuable technical help of Bastian and Regina Lüer.

Funding. No funding was received for this work.

Conflict of Interest. The authors declared no competing interests for this work.

Author Contributions. T.M. and C.v.H. wrote the manuscript. T.M. and C.v.H. designed the research. T.M. and C.v.H. performed the research. T.M. and C.v.H. analyzed the data.

1. Pascual, J., Zamora, J. & Pirsch, J.D. A systematic review of kidney transplantation from expanded criteria donors. *Am. J. Kidney Dis.* **3**, 553–586 (2008).
2. Ojo, A.O. Expanded criteria donors: process and outcomes. *Semin. Dial.* **6**, 463–468 (2005).
3. Aufricht, C. Heat-shock protein 70: molecular supertool? *Pediatr. Nephrol.* **6**, 707–713 (2005).
4. Chebotareva, N., Bobkova, I. & Shilov, E. Heat shock proteins and kidney disease: perspectives of HSP therapy. *Cell Stress Chaperones* **3**, 319–343 (2017).
5. Kalmar, B. & Greensmith, L. Induction of heat shock proteins for protection against oxidative stress. *Adv. Drug Deliv. Rev.* **4**, 310–318 (2009).
6. O'Neill, S., Harrison, E.M., Ross, J.A., Wigmore, S.J. & Hughes, J. Heat-shock proteins and acute ischaemic kidney injury. *Nephron Exp. Nephrol.* **4**, 167–174 (2014).
7. Guo, Q. *et al*. Ischemic postconditioning prevents renal ischemia reperfusion injury through the induction of heat shock proteins in rats. *Mol. Med. Rep.* **6**, 2875–2881 (2014).
8. von Horn, C. & Minor, T. Improved approach for normothermic machine perfusion of cold stored kidney grafts. *Am. J. Transl. Res.* **10**, 1921–1929 (2018).
9. Minor, T. & von Horn, C. Rewarming injury after cold preservation. *Int. J. Mol. Sci.* **9**, 2059 (2019).
10. Minor, T., von Horn, C. & Paul, A. Role of erythrocytes in short-term rewarming kidney perfusion after cold storage. *Artif. Organs* **6**, 584–592 (2019).
11. Brezis, M., Heyman, S.N. & Epstein, F.H. Determinants of intrarenal oxygenation. II. Hemodynamic effects. *Am. J. Physiol.* **6**(Pt 2), F1063–F1068 (1994).
12. Calzavacca, P. *et al*. Long-term measurement of renal cortical and medullary tissue oxygenation and perfusion in unanesthetized sheep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **10**, R832–R839 (2015).
13. Qi, Z., Qi, S., Gui, L., Shen, L. & Feng, Z. Daphnetin protects oxidative stress-induced neuronal apoptosis via regulation of MAPK signaling and HSP70 expression. *Oncol. Lett.* **3**, 1959–1964 (2016).
14. Moers, C. *et al*. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N. Engl. J. Med.* **1**, 7–19 (2009).
15. Tingle, S.J. *et al*. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. *Cochrane Database Syst. Rev.* **3**, CD011671 (2019).
16. Hameed, A.M., Pleass, H.C., Wong, G. & Hawthorne, W.J. Maximizing kidneys for transplantation using machine perfusion: from the past to the future: a comprehensive systematic review and meta-analysis. *Medicine (Baltimore)* **40**, e5083 (2016).
17. Gallinat, A. *et al*. Role of pulsatility in hypothermic reconditioning of porcine kidney grafts by machine perfusion after cold storage. *Transplantation* **96**, 538–542 (2013).
18. Thuillier, R. *et al*. Benefits of active oxygenation during hypothermic machine perfusion of kidneys in a preclinical model of deceased after cardiac death donors. *J. Surg. Res.* **2**, 1174–1181 (2013).
19. Mazilescu, L.I. & Selzner, M. Kidney machine preservation: state of the art. *Curr. Transp. Reports* **3**, 234–241 (2019).
20. Hosgood, S.A., van Heurn, E. & Nicholson, M.L. Normothermic machine perfusion of the kidney: better conditioning and repair? *Transpl. Int.* **6**, 657–664 (2015).
21. Richter, K., Haslbeck, M. & Buchner, J. The heat shock response: life on the verge of death. *Mol. Cell* **2**, 253–266 (2010).
22. Moloney, T.C., Hoban, D.B., Barry, F.P., Howard, L. & Dowd, E. Kinetics of thermally induced heat shock protein 27 and 70 expression by bone marrow-derived mesenchymal stem cells. *Protein Sci.* **6**, 904–909 (2012).
23. Daugaard, M., Rohde, M. & Jaattela, M. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* **19**, 3702–3710 (2007).
24. Park, K.M., Kramers, C., Vayssier-Taussat, M., Chen, A. & Bonventre, J.V. Prevention of kidney ischemia/reperfusion-induced functional injury, MAPK and MAPK kinase activation, and inflammation by remote transient ureteral obstruction. *J. Biol. Chem.* **3**, 2040–2049 (2002).
25. Wang, Z. *et al*. Heat shock protein 72 protects kidney proximal tubule cells from injury induced by triptolide by means of activation of the MEK/ERK pathway. *Int. J. Toxicol.* **3**, 177–189 (2009).
26. Fujigaki, Y. *et al*. Immunohistochemical study of heat shock protein 27 with respect to survival and regeneration of proximal tubular cells after uranyl acetate-induced acute tubular injury in rats. *Ren. Fail.* **1**, 119–125 (2010).
27. Kaths, J.M. *et al*. Continuous normothermic ex vivo kidney perfusion is superior to brief normothermic perfusion following static cold storage in donation after circulatory death pig kidney transplantation. *Am. J. Transplant.* **4**, 957–969 (2017).
28. Nicholson, M.L. & Hosgood, S.A. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am. J. Transplant.* **5**, 1246–1252 (2013).
29. Minor, T. *et al*. First-in-man controlled rewarming and normothermic perfusion with cell-free solution of a kidney prior to transplantation. *Am. J. Transplant.* **4**, 1192–1195 (2020).
30. Maack, T. Physiological evaluation of the isolated perfused rat kidney. *Am. J. Physiol.* **2**, F71–F78 (1980).
31. David, R.T. The isolated perfused rat kidney model: a useful tool for drug discovery and development. *Curr. Drug Discov. Technol.* **1**, 97–111 (2004).
32. Cuypers, Y. *et al*. The functional state of the isolated rabbit kidney perfused with autologous blood. *Pflugers Arch.* **4**, 634–642 (2000).
33. Yonezawa, K. *et al*. Suppression of tumor necrosis factor-alpha production and neutrophil infiltration during ischemia-reperfusion injury of the liver after heat shock preconditioning. *J. Hepatol.* **5**, 619–627 (2001).
34. Kim, M. *et al*. Selective renal overexpression of human heat shock protein 27 reduces renal ischemia-reperfusion injury in mice. *Am. J. Physiol. Renal Physiol.* **2**, F347–F358 (2010).
35. Wu, H., Lai, C.-F., Chang-Panesso, M. & Humphreys, B.D. Proximal tubule translational profiling during kidney fibrosis reveals proinflammatory and long noncoding RNA expression patterns with sexual dimorphism. *J. Am. Soc. Nephrol.* **1**, 23–38 (2020).