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Original Article

Improved approach for normothermic machine perfusion of cold stored kidney grafts

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Abstract: Normothermic machine perfusion can decrease reperfusion injury in renal transplantation. Clinical procurement logistics include retrieval and initial transport of the graft using static cold storage. Therefore, use and benefits of brief normothermic reconditioning by machine perfusion should be investigated in the initially cold preserved graft. Porcine kidneys (6 per group) were retrieved 20 min after cardiac standstill. After 20 h of static cold preservation some grafts were put on a machine perfusion circuit and normothermically perfused for 2 h at 35°C (NMP). Another group was subjected to controlled oxygenated rewarming (COR), starting perfusion at 8°C and elevating temperature and pressure slowly up to 35°C and 75 mmHg during the first 90 min of 2 h perfusion. Control kidneys were only cold stored (CS). Post implant graft function was evaluated afterwards in an established *in vitro* reperfusion model. During graft reconditioning, COR reduced oxygen free radical production and formation of 4-hydroxy-2-nonenal (HNE), an activator of mitochondrial uncoupling proteins, in comparison to NMP. Upon reperfusion, NMP only led to a slight improvement of renal function (clearance of creatinine, fractional excretion of Na and glucose) compared to controls. But 2-3 fold improvements of renal function were seen after COR, which also significantly improved aerobic efficiency (total Na absorption/VO_2) upon reperfusion. A slow and controlled increase in temperature up to normothermia improves mitochondrial recovery and oxygen utilization efficiency, resulting in better functional recovery, possibly through a more mild and adapted increase of cellular metabolism.

Keywords: Reperfusion injury, machine perfusion, rewarming

Introduction

Reperfusion injury after ischemic preservation represents one of the leading causes of renal dys- or non-function after transplantation. Severe shortage of donor organs, along with epidemiologic changes on the donor site like increased age, obesity and co-morbidities has led to an extension of accepted donor criteria up to and including organ grafts that were retrieved after circulatory standstill [1].

However, although functional reserve of these less than optimal organ grafts is reduced and render them much more vulnerable to ischemic alterations [2, 3], these organs actually represent an increasing part in daily transplant routine [2, 4].

In order to cope with the reduced resilience of these organs towards ischemic preservation, several approaches have been adopted aiming to improve post preservation viability of these grafts [5].

Thus, preservation by hypothermic machine perfusion has often shown superior results to static cold storage in experimental and also clinical studies [6, 7].

Machine perfusion of kidney grafts also offers some potential to serve as a tool for evaluation of kidney grafts in order to reduce the uncertainty with respect to graft viability often encountered when using marginal donor organs [8].

So far, a variety of biomarkers have been measured in machine perfusion perfusates. However all parameters were of only limited predictive value in large clinical trials [9] and should not be used in isolation for discard decisions [8].

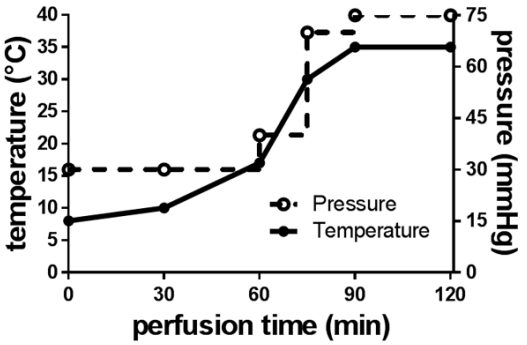

Figure 1. Controlled oxygenated rewarming: Gentle elevation of graft temperature and perfusion pressure upon extracorporeal oxygenated machine perfusion prior to warm reperfusion. Functional evaluation was done during constant perfusion at 35°C.

Similarly, rheological data and perfusion resistance at hypothermia could be significantly correlated to ulterior graft function in the recipient [7], but individual conclusions on post-transplant renal recovery remained very limited.

As a consequence, practical discard rate of marginal kidney grafts is expected to be higher than necessary due to precautious discard of questionable organs.

Over the last years increased attention has been spent to normothermic machine perfusion of donor kidneys [10, 11]. The proximity to physiological conditions is thought to improve pre-transplant evaluation of graft function and to offer potential for therapeutic approaches. Direct delivery of e.g. gene therapeutics (viral vectors) during NMP circumvents toxicity problems of systemic administration and enables a close monitoring [12].

However, due to the current practice of organ donation and transport logistics, clinical use of normothermic machine perfusion has so far only been undertaken after previous cold flush and hypothermic shipping of the grafts [13].

On the other hand, it has been shown that abrupt rewarming may foster mitochondrial dysfunction and tissue injury [14, 15].

In a rodent model, end-ischemic machine perfusion at hypothermia has shown less release of oxygen free radicals, less endothelial activation and lower levels of danger associated patterns (DAMPS's) than perfusion at normothermia [16].

Nonetheless, it appears that moderation of the temporal kinetics of warming up from hypothermia is likely to reduce the above described rewarming injury [17].

While previous studies were confined to perfusate temperatures between 8° and 20°C, the present study was undertaken to refine on suitability and benefits of brief normothermic reconditioning of the cold stored graft by *ex vivo* machine perfusion with either abrupt or gentle rise in temperature during perfusion.

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Kidneys were removed from dead German Landrace pigs weighing between 25 and 30 kg.

Twenty min after cardio-circulatory standstill, the renal artery was cannulated and the kidneys flushed by 100 cm gravity with 100 ml of UW-solution (Bridge to life, London, UK) on the back-table at 4°C. After 20 h of static cold preservation in UW solution the grafts were randomly assigned to one of the following groups (n=6, resp.):

1) Additional end-ischemic pulsatile normothermic machine perfusion (NMP) for 2 hours. NMP was effectuated based on the technique described by Kaths et al. [11] at 75 mmHg with STEEN solution (XVivo, Göteborg, Sweden) (300 ml, diluted 1/1 with Ringer's solution). The perfusate was supplemented with 5 IE insulin, 2.2 ml calcium gluconate 10%, 6.6 ml sodium bicarbonate 8.4%, 5000 IE heparin and 100 ml autologous washed erythrocytes (<1 leukocyte/nL). Perfusate was oxygenated with 95% Oxygen and 5% Carbon dioxide by means of a hollow fiber oxygenator placed in the arterial line of the circuit. Verapamil was given as single dose as required to maintain renal flow.

2) Additional end-ischemic machine perfusion for 2 hours with diluted STEEN solution starting at 8°C and controlled oxygenated rewarming (COR) of the perfusate in a hyperbolic pattern from 8°C to 35°C during the first 90 min, accompanied by an adapted pressure increase

from 30 to 75 mmHg. Autologous erythrocytes were only added after temperature has reached 20°C. The last 30 min of perfusion were kept constant at 35°C and used for evaluation of renal function (**Figure 1**).

3) A control group was done without further treatment (control).

Thereafter all grafts were flushed with 100 ml of cold saline solution and exposed to no flow conditions at room temperature for 20 min in order to imitate the time of surgical engraftment.

Reperfusion model

Functional recovery of the grafts was tested using an established *in vitro* model as described previously [18]. In brief, kidneys were put into a moist chamber and perfused at 37°C with 1000 ml Krebs-Henseleit buffer to which were added 2.2% bovine serum albumin and 20 ml of concentrated amino acid solution (RPMI 1640-50x)

Perfusate was oxygenated with a mixture of 95% oxygen and 5% carbon dioxide by a hollow fibre oxygenator (Hilite LT 1000, Medos, Stolberg, Germany) and supplemented with 0.1 g/l of creatinine to allow for calculation of renal clearances. Cannulation of the ureter was performed with PE-tubing for urine collection throughout the reperfusion period.

Kidney perfusion pressure was set at 90 mmHg and automatically maintained by a servo-controlled roller-pump, connected to a pressure sensor placed in the inflow line immediately prior to the renal artery.

Urine loss was replaced by adding equal amounts of balanced salt solution to the perfusion medium.

Analytical procedures

Concentrations of lactic acid dehydrogenase (LDH) and creatinine were determined in a routine fashion at the Laboratory centre of the University Hospital.

Clearances were calculated for the respective intervals as urinary creatinine x urine flow/perfusate creatinine.

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

Oxygen consumption (VO_2) was calculated from the differences between arterial and venous sites and expressed as μmol min^1 g^1 according to trans-renal flow and kidney mass.

Fractional excretion of sodium (FE Na) or glucose (FE glu) have been calculated as additional criteria for the vitality of renal proximal tubules according to:

$$FE\ Na/glu = Na/glu_{(urine)} \times Creatinine_{(perfusate)} / Na/glu_{(perfusate)} \times Creatinine_{(urine)} \times 100.$$

As sodium reabsorption represents the major energy consuming process in the kidney, the efficiency of renal O_2 utilization was approximated by the ratio of total kidney transport of Na (TNa) and VO_2, with TNa being equal to filtered Na minus excreted Na:

TNa=(GFR × perfusate Na)-(urinary UNa × urine flow) [19].

Oxygen free radical mediated lipid peroxidation (LPO) was evaluated by fluorimetry using the adduct formation with thiobarbituric acid as detailed elsewhere [20].

Measurements of neutrophil gelatinase associated lipocalin (NGAL) were done with a commercialized ELISA kit (USCN life science, Wuhan, China) according to the instructions of the manufacturer on a fluorescence micro plate reader (Tecan, Grailsheim, Germany).

Detection of 4-hydroxy-2-nonenal (HNE)

Frozen tissue samples were homogenized in T-Per tissue protein extraction reagent (Thermo Scientific) containing protease and phosphatase inhibitors (Thermo Scientific). Total protein concentration of tissue lysate was analyzed by BCA protein assay (Thermo Scientific) and calculated using a BSA standard curve. 10 μg of protein sample were transferred to a gel and an electrophoresis run was performed. Afterwards protein bands were blotted on a nitrocellulose membrane with a Transblot Turbo Transfer System (BioRad). The membrane was blocked in TBS (BioRad) with 1% Tween 20 and 5% milk powder.

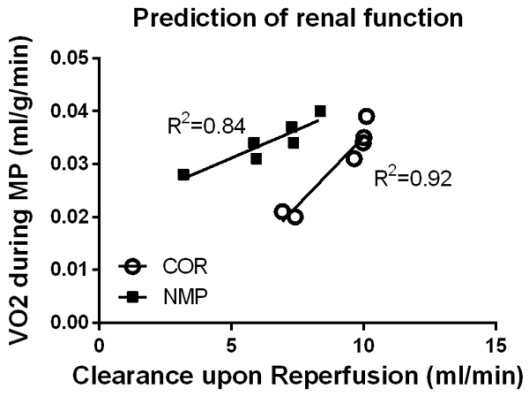

Figure 2. Correlation between renal oxygen consumption during reconditioning machine perfusion at 35°C after 20 h of cold storage in UW either by 2 h of normothermic machine perfusion at 35°C (NMP) or by controlled oxygenated rewarming by machine perfusion while elevating perfusate temperature from 8°C to 35° during the first 90 min of perfusion (COR) and ulterior renal function (overall clearance of creatinine) observed upon isolated reperfusion in vitro.

Expression of HNE was evaluated using overnight incubation at 4°C with polyclonal antibody (1/100, abcam, Cambridge, UK). Secondary antibody goat anti-rabbit IgG (Li-COR) was diluted 1:10000 in blocking buffer and membrane was incubated at room temperature for 1 hour. Target proteins were detected by enhanced chemiluminescence (ECL) using a C-Digit Blot Scanner (Li-COR).

Monoclonal anti-actin antibody was used for normalization. Quantification of protein content was performed densitometrically with UN-SCAN-IT gel v 6.1 (Silk Scientific Corporation, Orem UT).

Gene expression analyses

Total RNA was isolated from snap frozen samples using RNeasy® Mini Kit. Equal amounts of RNA were quantified by NanoQuant® (Tecan Infinite M200pro), complementary DNA synthesized by incubation with RT^2 First Strand Kit. The PCR reaction mix was prepared by using RT^2 SYBR Green Master Mix followed by amplification in a CFX96 thermocycler (Bio-Rad, Düsseldorf, Germany. All samples were assayed in triplicate using a multiplex PCR array (Qiagen).

The amount of specific mRNA in the tissue was normalized for the respective individual quantities of transcripts of GAPDH, which was analysed as house-keeping gene.

Results are expressed as relative deviation from baseline levels that were analysed from native cortical kidney samples processed in parallel.

All reagents and primers for GAPDH (n°PPS-00192A), OPA1 (n°PPS14195A), Fis1 (n°PPS-17019A) and COX18 (n°PPS13283A) were purchased from Qiagen GmbH (Hilden, Germany).

Statistics

All values were expressed as means ± SD. After proving the assumption of normality, differences between the groups were tested by one way ANOVA and post hoc testing with the Tukey Kramer test (Instat 3.01; Graph Pad software Inc, San Diego, CA), unless otherwise indicated. Statistical significance was set at $P<0.05$.

Results

Normothermic machine reconditioning prior to reperfusion

During machine perfusion, differences were seen between gradual or abrupt increase of temperature with regard to cellular injury parameters.

Thus, oxygen free radical induced lipid peroxidation (LPO: 0.30±0.13 vs 0.48±0.11 nmol/ml; COR vs NMP, $P<0.05$) was found to be significantly reduced after COR. Likewise, tubular cell injury as judged by the release of neutrophil gelatinase associated lipocalin (NGAL), amounted to only 3.4±2.3 ng/ml at the end of COR but 7.0±1.6 ng/ml after NMP, $P<0.05$).

On the other hand, irrespective of the fact, if reconditioning perfusion was started immediately at 35° or gradually increased from 8° to 35°C, a good correlation was seen between renal oxygen consumption at the end of machine perfusion at 35°C and ulterior glomerular filtration rate upon reperfusion, as expressed by the clearance of creatinine (cf. **Figure 2**).

No valuable correlation was found for other parameters readily available during machine perfusion like vascular resistance or release of LDH into the perfusate, both of which only achieved correlations below $r^2=0.4$.

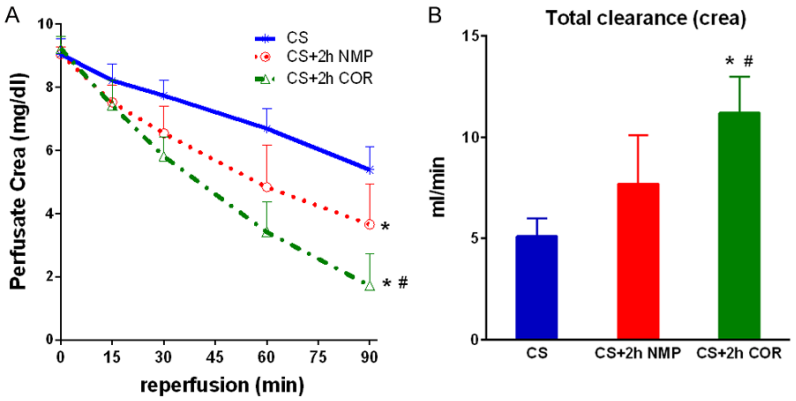

Figure 3. A. Removal of creatinine from the circulating perfusate over time during isolated reperfusion in vitro after 20 h of cold storage in UW (CS) or after 2 h of subsequent reconditioning by either normothermic machine perfusion at 35°C (NMP) or by controlled oxygenated rewarming by machine perfusion while elevating perfusate temperature from 8°C to 35° during the first 90 min of perfusion (COR). B. Total clearance of creatinine calculated over the entire reperfusion period. (*: P<0.05 vs CS; #: P<0.05 vs CS+2h NMP).

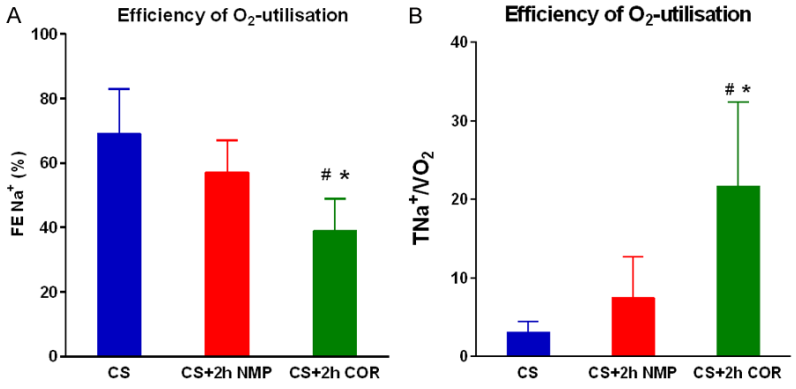

Figure 4. Approximation of tubular cell function upon isolated reperfusion in vitro after 20 h of cold storage in UW (CS) or after 2 h of subsequent reconditioning by either normothermic machine perfusion at 35°C (NMP) or by controlled oxygenated rewarming by machine perfusion while elevating perfusate temperature from 8°C to 35° during the first 90 min of perfusion (COR). A. Fractional excretion of sodium (FENa); B. Efficiency of oxygen utilization (ratio of total sodium reabsorption and oxygen consumption) upon isolated reperfusion in vitro. (*: P<0.05 vs CS; #: p<0.05 vs CS+2h NMP).

Postischemic recovery upon reperfusion

Renal glomerular function upon reperfusion was positively affected by either method of end-ischemic reconditioning, as compared to simple cold storage alone (cf. **Figure 3**).

However, elimination of creatinine from the circulating perfusate was most effective after COR resulting in significantly lower terminal creatinine levels than observed in the NMP or CS groups. Accordingly, clearance values that were calculated for the entire reperfusion period were found to be significantly enhanced after COR (cf. **Figure 3B**).

Tubular transport function was evaluated by calculating the fractional excretion of sodium (FE Na). In line with the data on glomerular filtration a significant improvement of FE Na was seen after end-ischemic reconditioning by COR as compared to NMP or CS alone (**Figure 4A**). Similar data were also observed with regard to fractional excretion of glucose which amounted to 100±15%, 65 ±17%* and 37±14%*,# after CS, CS+2h NMP and CS+2h COR, resp.; *: P<0.05 vs CS, #: P<0.05 vs CS+2h NMP).

The efficiency of oxygen utilization by the renal tissue is fairly well approximated by the ratio of total sodium transport (TNa) and the corresponding oxygen consumption (VO_2).

Of note, TNa/VO_2 was by far most preserved after circumventing the abrupt transit from cold to warm by COR, while oxygen utilization efficiency was seen significantly hampered in both other groups (**Figure 4B**).

As a reference, normal values for TNa/VO_2 ratio in our model amounted to 27±17, calculated from data of previously performed in vitro experiments on non-ischemic porcine kidneys.

In search for a possible reason for mitochondrial uncoupling we were looking for tissue expression of hydroxynonenal (HNE), a peroxidation product of membrane phospholipids, physiological concentrations of which can induce proton leaks at the inner mitochondrial membrane [21]. It was seen, that protein expression of HNE was significantly reduced after

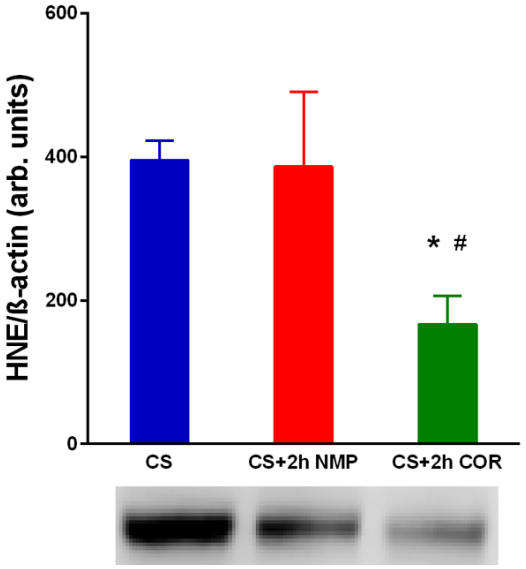

Figure 5. Tissue levels of 4-hydroxy-2-nonenal (HNE) at the end of isolated reperfusion in vitro after 20 h of cold storage in UW (CS) or after 2 h of subsequent reconditioning by either normothermic machine perfusion at 35°C (CS+2h NMP) or by controlled oxygenated rewarming by machine perfusion while elevating perfusate temperature from 8°C to 35° during the first 90 min of perfusion (CS+2h COR). Quantification performed by densitometric analysis of western blot images and normalization to individual expression of ß-actin. A representative blot picture of HNE is shown below. Data are given in arbitrary units. (*: P<0.05 vs CS; #: P<0.05 vs CS+2h NMP).

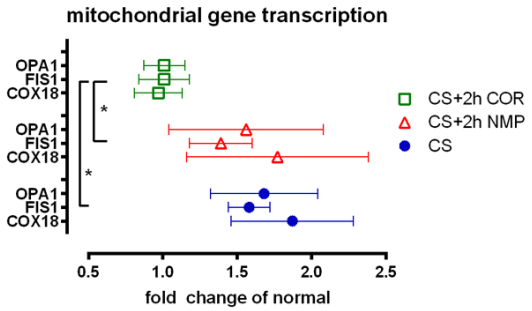

Figure 6. Alteration of mitochondrial mRNA expression upon reperfusion of isolated kidneys after 20 h of cold storage in UW (CS) or after 2 h of subsequent reconditioning by either normothermic machine perfusion at 35°C (CS+2h NMP) or by controlled oxygenated rewarming by machine perfusion while elevating perfusate temperature from 8°C to 35° during the first 90 min of perfusion (CS+2h COR). Upregulation is shown for dynamin like GTPase (OPA1), Fission protein 1 (FIS1) and Cytochrome oxidase assembly factor (COX18), as genes involved in mitochondrial repair and function, normalized to GAPDH as housekeeping gene. Means and SD of changes in gene expression in relation to normal levels evaluated from healthy kidneys without any ischemic challenge are depicted for each group. (*: P<0.05 between groups).

COR, while NMP had no major influence on the formation of HNE upon reperfusion (**Figure 5**).

Mitochondrial alteration upon reperfusion was also looked after following gene expression of specific genes involved in mitochondrial structure and function (cf. **Figure 6**). All genes under investigation (OPA1, Fis1 and COX18) showed an approximately 50% upregulation in comparison to baseline values in the CS group and similar results were disclosed in the CS+2h NMP group. By contrast, CS+2h COR resulted in a notably mitigated dys-balance of post-ischemic gene regulation, which remained virtually identic to baseline conditions.

Discussion

The results of the present study indicate, that a delayed restoration of final perfusion temperature effectively improves the functional recovery after ex vivo normothermic machine perfusion of hypothermically preserved renal grafts. A controlled increase in perfusion temperature (COR) results in graded recovery of metabolic function and seems to be operative in limiting metabolic charge during the most vulnerable period of reoxygenation.

Under physiologic conditions oxygen is reduced at the inner mitochondrial membrane (IMM) through the electron transport chain, which is composed of five enzyme complexes. Oxygen depletion results in accumulation of the citric acid cycle intermediate succinate, previously determined as universal indicator of tissue ischemia [22]. Succinate suppresses NADP(H) oxidation, but instead drives extensive superoxide production upon rapid re-oxidation by retrograde electron transport at the mitochondrial complex I (NADH dehydrogenase) [22].

Superoxide and related reactive oxygen species (ROS) induce the peroxidation of membrane phopsholipids and the production of injurious radicals [23]. The mechanisms behind complex I related ROS release and initial mitochondrial energy failure are unambiguously considered largely responsible for reperfusion injury after longer ischemia of organs [24]. In this context it is of interest that COR was operative in significantly reducing hallmarks of radical mediated lipid peroxidation during *ex vivo* machine perfusion in comparison to NMP.

The initial burst of ROS formation seems to be mitigated by hypothermia induced depression of metabolic activity during the early period of reoxygenation. However, the main finding of our study was that the use of a pressure and temperature controlled perfusion model largely preserved normal efficiency of oxygen utilisation in the tissue also during later reperfusion.

Although not directly shown in the present study, it is suggested, that uncoupling processes at the mitochondrial oxidative phosphorylation partly account for the reduced aerobic efficiency of abruptly rewarmed kidneys.

In isolated mitochondrial preparations it could be shown that exposure to elevated concentrations of exogenous or endogenous ROS is likely to induce uncoupling proteins (UCP) at the IMM. UCPs separate oxidative phosphorylation from ATP sythesis through proton release across the membrane. This mechanism is a common pathway to decrease endogenous superoxide production at the mitochondrium [25].

The mitochondrial uncoupling, however, results in an increased but partly futile renal VO_2 and thus reduced TNa/VO_2 ratio [26]. Recently, hydroxynonenal (HNE) has been identified as a radical peroxidation product at the mitochondrial membrane that induces proton leaks at inner mitochondrial membrane and mediates activation of uncoupling protein 2 [21].

Interestingly, the formation of HNE in our study could be reduced by ex vivo perfusion starting at hypothermic temperatures instead of abrupt normothermic perfusion.

Previous studies have also shown that mitochondrial injury is followed by post-ischemic repair processes, that are initiated by upregulation of mRNA expression of proteins related to mitochondrial function and structure [27, 28]. In this context is of interest that COR largely prevented such dys-homeostasis of mitochondrial gene expression upon reperfusion, which was however present in the two other groups in the present investigation.

Our current findings antagonize limitations in the application of NMP and open new perspectives. It has been shown in kidneys and livers that preceding periods of cold ischemia can actually abrogate the beneficial effects of normothermic preservation [29, 30]. This can be linked to a quick retransformation of complex I, which is physiologically deactivated upon lack of oxygen, into its active form upon normothermic re-exposure to oxygen, ensuing in a burst of respiration at not yet balanced metabolic intermediates [24]. Transient inhibition of complex I during postischemic reperfusion is thus proposed to protect mitochandrial integrity and to decrease ischemia reperfusion injury [31], and hypothermia during initial perfusion upon COR might well be operative in slowing down complex I activation.

In face of the increasing need to use grafts of reduced quality for transplantation, in house normothermic *ex-vivo* perfusion becomes an attractive tool that provides opportunities to improve regeneration [12], molecular or genetic *ex vivo* therapy and functional viability testing [32] of the isolated graft prior to implantation. By much more approaching conditions of physiological metabolism than reconditioning measures restricted to hypothermia [33], normothermic perfusion appears to be more promising for pharmacodynamic interventions and functional evaluation of the organ.

In our model, COR as well as NMP did allow for evaluation of pre-transplant graft viability, with good correlations of e.g. oxygen consumption during COR or NMP to ulterior renal function during reperfusion.

Adequate management of the rewarming process, that will mitigate 'rewarming injury' and mitochondrial dysfunction could possibly make up for the relatively poorer benefit of NMP after CS as compared to continuous NMP [29] but this has to be confirmed in future studies.

The logistic advantage of COR after transport of the organ by simple cold storage, however, is evident. The concept does not postulate the availability of a portable normothermic kidney perfusion device, nor trafficing of machines to and from the explant hospital and minimizes the risk of organ loss due to machine failure during normothermic shipping.

The conclusions drawn from this study albeit are subject to certain limitations which are inherent to the *in vitro* model used.

Thus, vascular interactions with cellular blood components upon reperfusion *in vivo* are not taken into account. Therefore, post-ischemic

inflammatory reactions, and especially the additional protective effect of normothermic pre-perfusion with plasma and leucocyte-free media [13], will be underestimated in our study. Nonetheless, the direct comparison of controlled versus abrupt warming up will not be affected by this drawback.

Although early functional recovery and non-immunological tissue injury incurred upon reperfusion can be reliably evaluated in our model [34], the data do not allow for direct conclusions on long term graft function. Therefore, subsequent studies involving outcome evaluation after transplantation *in vivo* are strongly encouraged.

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Disclosure of conflict of interest

None.

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References

[1] Matas AJ, Smith JM, Skeans MA, Thompson B, Gustafson SK, Stewart DE, Cherikh WS, Wainright JL, Boyle G, Snyder JJ, Israni AK and Kasiske BL. OPTN/SRTR 2013 annual data report: kidney. Am J Transplant 2015; 15 Suppl 2: 1-34.

[2] Watson CJ, Wells AC, Roberts RJ, Akoh JA, Friend PJ, Akyol M, Calder FR, Allen JE, Jones MN, Collett D and Bradley JA. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. Am J Transplant 2010; 10: 1991-1999.

[3] Maathuis MH, de GM, Ploeg RJ and Leuvenink HG. Deterioration of endothelial and smooth muscle cell function in DCD kidneys after static cold storage in IGL-1 or UW. J Surg Res 2009; 152: 231-237.

[4] Pascual J, Zamora J and Pirsch JD. A systematic review of kidney transplantation from expanded criteria donors. Am J Kidney Dis 2008; 52: 553-586.

[5] Hoffmann T and Minor T. New strategies and concepts in organ preservation. Eur Surg Res 2015; 54: 114-126.

[6] Moers C, Pirenne J, Paul A and Ploeg RJ. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2012; 366: 770-771.

[7] Jochmans I, O'Callaghan JM, Pirenne J and Ploeg RJ. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. Transpl Int 2015; 28: 665-676.

[8] Parikh CR, Hall IE, Bhangoo RS, Ficek J, Abt PL, Thiessen-Philbrook H, Lin H, Bimali M, Murray PT, Rao V, Schroppel B, Doshi MD, Weng FL and Reese PP. Associations of perfusate biomarkers and pump parameters with delayed graft function and deceased donor kidney allograft function. Am J Transplant 2016; 16: 1526-1539.

[9] Moers C, Varnav OC, van HE, Jochmans I, Kirste GR, Rahmel A, Leuvenink HG, Squifflet JP, Paul A, Pirenne J, van OW, Rakhorst G and Ploeg RJ. The value of machine perfusion perfusate biomarkers for predicting kidney transplant outcome. Transplantation 2010; 90: 966-973.

[10] Hosgood SA, Nicholson HF, Nicholson ML. Oxygenated kidney preservation techniques. Transplantation 2012; 93: 455-459.

[11] Kaths JM, Echeverri J, Goldaracena N, Louis KS, Chun YM, Linares I, Wiebe A, Foltys DB, Yip PM, John R, Mucsi I, Ghanekar A, Bagli DJ, Grant DR, Robinson LA and Selzner M. Eight-hour continuous normothermic Ex vivo kidney perfusion is a safe preservation technique for kidney transplantation: a new opportunity for the storage, assessment, and repair of kidney grafts. Transplantation 2016; 100: 1862-1870.

[12] Hosgood SA, van Heurn E and Nicholson ML. Normothermic machine perfusion of the kidney: better conditioning and repair? Transpl Int 2015; 28: 657-664.

[13] Nicholson ML and Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. Am J Transplant 2013; 13: 1246-1252.

[14] Leducq N, Delmas-Beauvieux MC, Bourdel-Marchasson I, Dufour S, Gallis JL, Canioni P and Diolez P. Mitochondrial permeability transition during hypothermic to normothermic reperfusion in rat liver demonstrated by the protective effect of cyclosporin A. Biochem J 1998; 336: 501-506.

[15] Minor T, von Horn C and Paul A. Role of temperature in reconditioning and evaluation of

cold preserved kidney and liver grafts. Curr Opin Organ Transplant 2017; 22: 267-273.
[16] Kron P, Schlegel A, de Rougemont O, Oberkofler CE, Clavien PA and Dutkowski P. Short, cool, and well oxygenated - HOPE for kidney transplantation in a rodent model. Ann Surg 2016; 264: 815-822.
[17] Minor T, Efferz P, Fox M, Wohlschlaeger J and Lüer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. Am J Transplant 2013; 13: 1450-1460.
[18] von Horn C and Minor T. Isolated kidney perfusion: the influence of pulsatile flow. Scand J Clin Lab Invest 2018; 78: 131-135.
[19] Pei L, Solis G, Nguyen MT, Kamat N, Magenheimer L, Zhuo M, Li J, Curry J, McDonough AA, Fields TA, Welch WJ and Yu AS. Paracellular epithelial sodium transport maximizes energy efficiency in the kidney. J Clin Invest 2016; 126: 2509-2518.
[20] Minor T and Koetting M. Gaseous oxygen for hypothermic preservation of predamaged liver grafts: Fuel to cellular homeostasis or radical tissue alteration? Cryobiology 2000; 40: 182-186.
[21] Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ and Brand MD. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. EMBO J 2003; 22: 4103-4110.
[22] Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord EN, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa AS, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T and Murphy MP. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 2014; 515: 431-435.
[23] Kalogeris T, Bao Y and Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol 2014; 2: 702-714.
[24] Drose S, Stepanova A and Galkin A. Ischemic A/D transition of mitochondrial complex I and its role in ROS generation. Biochim Biophys Acta 2016; 1857: 946-957.
[25] Murphy MP, Echtay KS, Blaikie FH, Asin-Cayuela J, Cocheme HM, Green K, Buckingham JA, Taylor ER, Hurrell F, Hughes G, Miwa S, Cooper CE, Svistunenko DA, Smith RA and Brand MD. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. J Biol Chem 2003; 278: 48534-48545.
[26] Hansell P, Welch WJ, Blantz RC and Palm F. Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. Clin Exp Pharmacol Physiol 2013; 40: 123-137.
[27] Van Itallie CM, Van Why S, Thulin G, Kashgarian M and Siegel NJ. Alterations in mitochondrial RNA expression after renal ischemia. Am J Physiol 1993; 265: C712-719.
[28] Stallons LJ, Funk JA and Schnellmann RG. Mitochondrial homeostasis in acute organ failure. Curr Pathobiol Rep 2013; 1.
[29] Kaths JM, Cen JY, Chun YM, Echeverri J, Linares I, Ganesh S, Yip P, John R, Bagli D, Mucsi I, Ghanekar A, Grant DR, Robinson LA and Selzner M. 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 2017; 17: 957-969.
[30] Reddy SP, Bhattacharjya S, Maniakin N, Greenwood J, Guerreiro D, Hughes D, Imber CJ, Pigott DW, Fuggle S, Taylor R and Friend PJ. Preservation of porcine non-heart-beating donor livers by sequential cold storage and warm perfusion. Transplantation 2004; 77: 1328-1332.
[31] Chouchani ET, Pell VR, James AM, Work LM, Saeb-Parsy K, Frezza C, Krieg T and Murphy MP. A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. Cell Metab 2016; 23: 254-263.
[32] Sutton ME, op den Dries S, Karimian N, Weeder PD, de Boer MT, Wiersema-Buist J, Gouw AS, Leuvenink HG, Lisman T and Porte RJ. Criteria for viability assessment of discarded human donor livers during ex vivo normothermic machine perfusion. PLoS One 2014; 9: e110642.
[33] Minor T and Paul A. Hypothermic reconditioning in organ transplantation. Curr Opin Organ Transplant 2013; 18: 161-167.
[34] Gallinat A, Fox M, Luer B, Efferz P, Paul A and Minor T. Role of pulsatility in hypothermic reconditioning of porcine kidney grafts by machine perfusion after cold storage. Transplantation 2013; 96: 538-542.