Objectives. Ischemic injury to the renal allograft prior to implantation is considered as the major cause of primary non and never-function (PNF) and delayed graft function (DGF). Evidence has been put forward that brain dead and non-heart-beating (NHB) donor organs are of marginal quality compared to living donors. The purpose of this study was to evaluate renal function and injury of brain dead and NHB donor kidneys using the isolated perfused rat kidney. Material and Methods. Fisher F344 rats were either maintained brain death for 4 hr or subjected to cardiac arrest for 45 min (NHB). Living rats served as controls. To omit additional effects of cold ischemia, kidneys were immediately reperfused. Renal function and injury were assessed by monitoring urine production, glomerular filtration rate (GFR), Na⁺ and K⁺ reabsorption, glucose metabolism and reabsorption, as well as release of brush border, lysosomal, and intracellular enzymes.

Results. Renal dysfunction and injury were most pronounced in NHB donor kidneys reflected by a highly reduced urine production, anaerobic glucose metabolism resulting in lactate formation, and significant higher luminal release of intracellular and lysosomal enzymes. Brain dead kidneys showed an increased urine production and were functionally abnormal in K⁺ reabsorption showing a net excretion of K⁺, probably as a result of ATP depletion. Loss of brush border occurred during brain death and cardiac arrest. Conclusions. Both, brain death and cardiac arrest have deleterious effects on renal function and renal injury. The ischecmically injured NHB donor kidney was functionally inferior compared to the brain dead donor kidney and living donor kidneys. However, both brain dead and NHB kidneys showed considerable renal damage compared to kidneys from living donors.

Keywords: Glomerular filtration rate, Reabsorption, Alanine aminopeptidase, Alkaline phosphatase, N-acetyl-β-D-glucosaminidase

Expansion of the donor pool is needed to meet the required number of donor organs in kidney transplantation. Despite an increasing number of living donations and a consistent number of brain dead donors the waiting lists for kidney transplantation are increasing. The use of non-heart-beating (NHB) donors may enlarge the donor pool significantly [1]. Before the actual transplant procedure in the recipient, a number of processes occur that are potentially hazardous for the graft-to-be. In case of the optimal donor, i.e. the living donor, injury is minimal. A short warm ischemic period during the explantation is followed by short cold ischemia time during preservation. In case of an agonizing period of brain death in cadaveric donors, many detrimental processes occur that lead to an immunologically activated organ, which after explantation will suffer even more due to a prolonged cold preservation time [2,3]. NHB donors, instead, are exposed to a prolonged warm ischemia period. With increasing warm ischemia time, when circulation has stopped and tissue becomes hypoxic, a progressive
damage is inflicted to the organ [4].

NHB kidney donors are categorized in 4 groups according to the Maastricht criteria [5]. Category I: patients who are dead on arrival. Category II: patients who had unsuccessful resuscitation. Category III: patients awaiting cardiac arrest, as in a ventilator switch off procedure. Category IV: patients who have a cardiac arrest during brain death. Of the NHB kidneys, nowadays, predominantly category III donors are used. They still represent a small percentage (<5%) of the potential NHB pool, but in contrast to category II donors, warm ischemia time in category III donors can be controlled. Nevertheless, NHB category III donor kidneys have higher rates of primary non and never-function (PNF; 4-14%) or delayed graft function (DGF; 40-80%) than living or brain dead donors [6-10]. In brain dead donors, DGF is a risk factor for inferior outcome. Three months post transplant, a persistent reduction of 10-15% in graft survival with DGF versus 4-6% reduction without DGF has been observed [11,12]. Despite a higher rate of initial poor function in the NHB donor kidney, graft survival of brain dead and NHB donor kidneys is comparable, and no pertinent effect of DGF in graft survival is seen [6-10].

The aim of the present study was to compare the physiological effects of brain death and of cardiac arrest with warm ischemia on renal function and nephron injury, to understand the setting in DGF. Since renal function can be affected by neural as well as humoral interactions in-vivo, we have studied functionality and viability of living, brain dead, and NHB kidneys in an isolated perfused kidney (IPK) set-up in the rat.

Materials and Methods

Experimental design
All animals received care in compliance with the guidelines of the Local Care and Use Committee following National Institutes of Health guidelines. Inbred adult male Fisher F344 rats, weighing 250-300 g, were used throughout the experiments. Animals were divided in a living, brain death, and non-heart-beating (NHB) group. The living group served as control. In each group six animals were used.

Brain death model
Rats were anesthesized using isoflurane with a mixture of NO₂ and O₂ during brain death induction. They were intubated via a tracheostomy by a blunt-tipped cannula and mechanically respirated. The animals were ventilated on a small rodent ventilator (Zoovent, Triumph Technical Services Ltd, UK) with 4 liter/min O₂ in air for 30 min (stroke rate: 46 per min, inspiration/expiration: 1/2, peak inspiratory pressure: 16 mm Hg, mean inspiratory pressure: 6 mm Hg, and a positive end expiratory pressure: 0 mm Hg). Thereafter, they were ventilated with 0.5 liter/min O₂ in air until the end of the brain death period.

Brain death was induced as follows. A 1x1 mm hole was frontomedially, just lateral of the central vein, drilled through the skull. A no. 4 Fogarty catheter (Fogarty Arterial Embolectomy Catheter: 4F, Edwards Lifesciences Co., Irvine, CA) was inserted in the epidural space with the tip pointing caudally. To simulate an epidural haematoma leading to brain death, intracranial pressure was increased gradually by slow inflation of the balloon (16 µl saline per min) using a syringe pump (Terufusion, Termo Co., Tokyo, Japan). Inflation of the balloon was stopped after approximately 25 min, when the blood pressure reached normal levels after an initial hypotension. Just after stopping balloon inflation, a sharp rise of blood pressure was seen that is typical in brain death development. Brain death was confirmed 30 min after induction by dilated and fixed pupils, the absence of corneal reflexes, and an apnea test. The average balloon volume that consistently induced brain death was 420±20 µl. The balloon was kept inflated during the entire experiment. Blood pressure was monitored via a PE cannula (0.40x0.80mm) implanted in the left femoral artery. A blood pressure above 80 mm Hg was considered normotensive. When blood pressure dropped beneath 80 mm Hg, rats were infused with 10% hydroxyethyl starch (HAES) until normotension was reached. Body temperature, measured with a rectal thermoprobe (Mon-a-therm 6510, Mallinckrodt Medical, Inc., St. Louis, MO), was maintained at 37°C by means of a heating pad and lamp.

After 4 hr of brain death, isoflurane 0.5% anesthesia with a mixture of NO₂ and O₂ was used 10 min before the end of the brain death period to achieve full muscle relaxation in order to allow abdominal surgery.

Non-heart-beating model
In anesthesized rats, anti-coagulation was accomplished by injecting 250 U Heparin in 1 ml saline via the penile vein. Five minutes after heparinization,
laparotomy was performed through a midline incision. NHB was induced as follows. A thoracotomy was performed followed by induction of cardiac arrest by external compression of the heart (tamponade) for 5 min [13]. After induction of cardiac arrest, the aorta was closed using a vascular clamp rostral from the heart. The thoracotomy wound was covered with gauze, moistened with 0.9% NaCl. Cooling of the rat was prevented by using a heating lamp. After 45 min of NHB, nephrectomy was performed.

**Nephrectomy**

Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4 (143.6 mM Na+, 5.9 mM K+, 123.3 mM Cl–, 25.0 mM HCO3–, 2.5 mM Ca2+, 1.2 mM Mg2+, 1.2 mM H2PO4–, 1.2 mM SO42–) supplemented with 0.8 g/l of inulin, 50 g/l of bovine serum albumin (fraction V), 1.1 g/l of D-glucose, and 0.5 mM (final concentration) of the amino acids L-cysteine, L-glutamic acid, and L-glycine (all supplements were from Sigma, St. Louis, MO), as well as a University of Wisconsin (UW) solution, pH 7.4 [14] were used as flushing media at nephrectomy.

Kidneys were harvested by the following procedure. After opening the abdomen by laparotomy through a midline incision, the ureter of the right kidney was cannulated, and the aorta (rostral from the right renal artery) and superior mesenteric artery were ligated. Blood was removed from the kidneys by pre-flushing with 10 ml 37°C oxygenated KRB (to avoid potential effects of warm ischemia) and a flush of 10 ml 4°C UW via the aorta. Subsequently, kidneys were removed en-bloc and placed on ice. The aorta was opened through a caudal-to-rostral incision. Remaining blood was removed by flushing the kidneys once again with 5 ml 4°C UW, and a cannula was left in the renal artery. In both kidneys, UW was flushed out with 5 ml 4°C KRB. The right kidney was used in the isolated perfused kidney (IPK) system. The left kidney was cut in slices. The slices used for enzyme histochemistry were frozen in isopentane (-80°C) and stored at -80°C.

**Isolated perfused kidney (IPK) procedure**

**Perfusion fluid.** A cell-free KRB buffer, pH 7.4, was used as perfusion fluid. For each IPK experiment, 300 ml KRB buffer was filtered through 0.45 µm and 0.2 µm filters (Schleicher & Schuell, Dassel, Germany). To 222.5 ml KRB buffer, 0.5 mM (final concentration) of the amino acids L-cysteine, L-glutamic acid, and L-glycine were added (perfusion fluid A). To 77.5 ml KRB buffer, 2.5 ml of a 41.25 mM amino acid mixture solution (Sigma), reaching a final concentration of 0.5 mM of each amino acid present in the solution, and 0.5 mM L-cysteine (Sigma), which was not included in the solution, were added (perfusion fluid B). Amino acids were included to improve the kidney viability [15]. Both perfusion fluids A and B were recirculated by a peristaltic pump (Ismatec, Zürich, Switzerland), filtered through an in-line 5-µm pore size filter (Millipore, Ireland), oxygenated with a mixture of 95%O2/5%CO2 by a capillary module, and passed through a heated glass bubble trap to maintain the temperature at 37°C and avoid the formation of air bubbles. Temperature was regulated by a thermostatically-controlled waterbath (Julabo, Seelbach, Germany).

**IPK set-up.** The right kidney was placed in a moist temperature chamber and perfused via a cannula inserted in the renal artery. IPK experiments were separated in 3 periods: stabilization, experimental, and terminal period. In all periods, IPK was performed using a pressure-controlled perfusion.

**Stabilization period.** The kidney was perfused with 100 ml perfusion fluid A for 15 min. Kidney perfusion pressure was set at 102±1.3 mm Hg, resulting in a perfusate flow rate of 11.2±0.5 ml/min/gram kidney. From these parameters, the intrarenal vascular resistance (IRR) before the experimental period of IPK was calculated.

**Experimental period.** The kidney was perfused with 60 ml (living and NHB kidneys) or 65 ml (brain dead kidneys) perfusion fluid B for 90 min. Samples of urine and perfusate were collected at 15-min intervals throughout the experimental period. During this period, pH ranged from 7.25±0.01 to 7.32±0.01 and pO2 ranged from 82.5±1.7 to 88.1±1.4 kPa.

**Terminal period.** The kidney was perfused with 100 ml of fresh perfusion fluid A for 5 min. The IRR after the experimental period of IPK was determined by registration of the kidney perfusion pressure and perfusate flow rate. Thereafter, when the kidney was still perfused with the perfusion fluid, a small part of cortical tissue was taken for determination of ATP. The tissue was directly transferred to 1 ml solution containing 2 mM EDTA and 70% v/v ethanol, and stored at -80°C. Subsequently, perfusion was stopped and the remnant kidney was further cut in slices. The slices used for enzyme histochemistry were frozen in isopentane (-80°C) and stored at -80°C.
Biochemical determinations

Indicators of renal function and injury were analyzed in perfusate and urine. Lactate dehydrogenase (LDH) in the perfusate was analyzed in a routine fashion at the Laboratory Center of the University Hospital Groningen (Mega, Merck, Amsterdam, The Netherlands). LDH activity was expressed as U/min/gram kidney.

Urinary NAG was measured using a method based on hydrolysis of p-nitrophenyl-NAG (Sigma), 60 mM KH₂PO₄ pH 7.2 at 37°C for 2 hr. Assay blanks were run in which 10 μl of 60 mM KH₂PO₄ was added to the reaction mixture. Samples were analyzed for the amount of formed product photometrically at 405 nm.

Activity of the brush border enzymes alanine aminopeptidase (AAP) and alkaline phosphatase (AP) and of the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) in urine and perfusate were measured by colorimetric assays. Enzyme activity was measured by means of enzymatically conversion of a specific substrate recognized by AAP, AP, or NAG. One unit of enzyme activity (U) was defined as the amount of enzyme which released 1 μmol of product per minute. Enzyme activity in the urine/perfusate was corrected for the volume of collected urine/perfusate and weight of the kidney, and expressed as μU/min/ml/gram kidney (urine) or U/min/gram kidney (perfusate).

AAP activity was measured using a method based on hydrolysis of alanine-p-nitroanilide into p-nitroaniline. A 10 μl urine sample was incubated with 10 μl activation buffer (10 mM Tris HCl, pH 8.0, 1 mM CoCl₂) and 190 μl substrate solution (1.66 mM L-alanine-p-nitroanilide (Sigma), 60 mM KH₂PO₄, pH 7.2) at 37°C for 2 hr. Assay blanks were run in which 10 μl of 60 mM KH₂PO₄ was added to the reaction mixture. Samples were analyzed for the amount of formed product photometrically at 405 nm.

AP activity was measured using a method based on hydrolysis of p-nitrophenylphosphate into p-nitrophenol. A 5 μl urine sample was incubated with 186 μl 50 mM 2-amino-2-methyl-1,3-propanediol, pH 9.8 (Acros, Geel, Belgium), 5 μl 100 mM MgCl₂, and 10 μl substrate solution (p-nitrophenylphosphate (Acros), 10 mg/ml, 0.9% NaCl) at 37°C for 30 min. Reaction was stopped by adding 20 μl 1N NaOH. Samples were centrifuged at 960g for 10 min. AP activity was measured in 175 μl supernatant. Assay blanks were run in which 5 μl of a 0.9% NaCl solution was added to the reaction mixture. Samples were analyzed for the amount of formed product photometrically at 405 nm.

In the NAG activity assay, p-nitrophenyl-NAG (Sigma) was used as substrate. NAG activity measurements were performed following the instructions of HaemoProbe (Groningen, The Netherlands). Briefly, NAG released in urine or perfusate hydrolyzed p-nitrophenyl-NAG into p-nitrophenol in the presence of citrate buffer. After 60 min at 37°C, 1 M Na₂CO₃ was added to the mixture to terminate the reaction and to develop a yellow colour released from the hydrolyzed substrate. Samples were analyzed for the amount of formed product photometrically at 405 nm.

Concentrations of ATP in homogenized tissue samples were determined by a luminescence assay using the ATP Bioluminescence assay kit CLS II (Boehringer, Mannheim, Germany). Samples were diluted in a 100 mM phosphate buffer (pH 7.6-8.0). After one minute centrifugation at 1000g, 50 μl of luciferase was added to 50 μl of the supernatant and measured in an illuminescence apparatus (Lucy-1, Anthos Labtec instruments, Salzburg). ATP levels were corrected to the protein concentration of the sample. The Bio-Rad protein micro photospectrometric assay (Bio-Rad) was used at 620 nm.
Enzyme histochemistry
Frozen kidney tissue was sectioned at 4-6 µm and processed for histochemical staining of AAP and AP.

For AAP staining, sections were incubated with 0.1 M Tris-HCl, pH 7.6 for 2 min, stained in the dark with 0.1 M Tris-HCl, pH 7.5, containing 0.2 mg/ml L-alanine-4 methoxy-β-naphtylamide (Sigma) and 0.2 mg/ml Fast Blue BB salt for 20 min. Sections used as negative control were incubated without L-alanine-4 methoxy-β-naphtylamide. Sections were then rinsed with 0.1 M Tris-HCl, pH 7.6, counter stained with Mayers Haematoxylin solution (Merck, Darmstadt, Germany) for 20 min, and subsequently mounted in glycerine.

For AP staining, sections were incubated with 0.1 M Tris-HCl, pH 7.6 for 2 min, stained in the dark with 0.1 M Tris-HCl, pH 9.0, containing 0.2 mg/ml naphtol AS-MX disodium salt (Sigma) and 0.2 mg/ml Fast Red TR azo dye for 20 min. Sections used as negative control were incubated without naphtol AS-MX disodium salt. Sections were then rinsed with 0.1 M Tris-HCl, pH 7.6, counter stained with Mayers Haematoxylin solution (Merck) for 20 min, and subsequently mounted in glycerine. Slides were evaluated by light microscopy.

Statistical analysis
Statistical significance was ascertained using ANOVA with LSD-correction for multiple comparison or the two-tailed unpaired Student t-test when appropriate. Results are expressed as mean±SEM and were considered significant at P<0.05.

Results

Intrarenal vascular resistance during reperfusion in an isolated perfused kidney model

Figure 1 shows the intrarenal vascular resistance (IRR) in kidneys from living, brain dead, and NHB rats at the 15-min duration of stabilization and after the 90-min duration of reperfusion. IRR was significantly increased in the NHB group during the stabilization period. After the experimental period of isolated kidney perfusion, IRR was similar in all groups (living: 5.78±0.37 mm Hg/ml/min; brain death: 5.10±0.34 mm Hg/ml/min; NHB: 5.42±0.39 mm Hg/ml/min). In addition, in NHB kidneys, IRR after 90 min of reperfusion was significantly decreased compared to the IRR of the stabilization period.

Biochemical determinations
Physiological function of the kidneys from all groups after the 90-min duration of the experimental period are summarized in Table 1. The majority of glucose was aerobically metabolized in all groups, indicating that the supply of oxygen to the kidneys had been sufficient. In the NHB group, however, anaerobic metabolism was significantly higher than in the living group, suggesting that cardiac arrest had affected functional activity of the kidney. In addition, whereas ATP in the brain dead kidney was significantly lower than in kidneys from living rats (ATP in living and brain dead kidney, respectively: 1.48±0.23, 0.78±0.04 pmol/µg protein; *P<0.05 vs. living), no evidence of ATP depletion was observed in the NHB kidney.

<table>
<thead>
<tr>
<th>Table 1. Biochemical parameters reflecting renal function in living, brain dead, and non-heart-beating rat kidneys after 90 min isolated kidney perfusion</th>
<th>Living kidney</th>
<th>Brain Dead kidney</th>
<th>Non-Heart-Beating kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urine (µl/min/gram)</td>
<td>312±64</td>
<td>588±65*</td>
<td>71±43*</td>
</tr>
<tr>
<td>GFR (µl/min/gram)</td>
<td>158±23</td>
<td>246±19*</td>
<td>46±29*</td>
</tr>
<tr>
<td>Fractional Na+ reabsorption (%)</td>
<td>78.3±3.1</td>
<td>80.4±1.9</td>
<td>74.1±3.8</td>
</tr>
<tr>
<td>Fractional K+ reabsorption (%)</td>
<td>22.2±8.5</td>
<td>-18.5±6.8**</td>
<td>ND</td>
</tr>
<tr>
<td>Fractional glucose reabsorption (%)</td>
<td>78.4±2.9</td>
<td>85.9±2.2</td>
<td>ND</td>
</tr>
<tr>
<td>Aerobe metabolism (%)</td>
<td>93±1.2</td>
<td>91.8±0.6</td>
<td>88.6±1.3*</td>
</tr>
</tbody>
</table>

*P<0.05 vs. living
**P<0.01 vs. living
ND: not determined

1) n = 4 (parameters were determined in urine and perfusate)
2) n = 6 (parameter was determined in perfusate)
(1.15±0.16 pmol/µg protein). Significant differences were found in total urine production and the rate of inulin clearance (glomerular filtration rate (GFR)) in the brain dead and NHB groups compared to the living group. Total urine production and inulin clearance (GFR) were significantly higher in the brain dead group. Both functional parameters were significantly lower in the NHB group. Fractional Na+ reabsorption did not significantly differ between groups. Similar results were found for fractional glucose reabsorption in the living and brain dead groups. Fractional K+ reabsorption, however, appeared to be abnormal in brain dead kidneys. Instead of reabsorption, as was found for Na+, K+ was excreted in the brain dead group. During the experimental period of isolated kidney perfusion, fractional K+ reabsorption was decreased in the living group (15 min: 55.3±6.1%; 90 min: 22.2±8.5%) (Figure 2). K+ reabsorption was observed in the brain dead group during the first 15 min of reperfusion (9.9±7.0%), and was significantly lower than in the living group. After this period of reperfusion, K+ excretion (negative K+ reabsorption) was seen in the brain dead group only, reaching a maximum at 60 min of reperfusion (30 min: -8.0±6.9%; 60 min: -35.5±10.4%; 90 min: -18.5±6.8%) (Figure 2).

Renal injury parameters measured in perfusate and urine after the 90-min duration of the experimental period are summarized in Tables 2 and 3, respectively. Living and brain dead kidneys did not significantly differ in the vascular release of lactate dehydrogenase (LDH) and N-acetyl-β-D-glucosaminidase (NAG). NHB kidneys showed significantly higher LDH and NAG release than the kidneys from the living group (Table 2). The urine produced in the brain dead group showed significantly less activity of alanine amino peptidase (AAP), alkaline phosphatase (AP), and NAG than urine produced in the living group (Table 3). Total protein in the urine did not significantly differ between the living and brain dead groups.

Table 2. Biochemical parameters, determined in the perfusate, reflecting renal injury in living, brain dead, and non-heart-beating rat kidneys after 90 min isolated kidney perfusion

<table>
<thead>
<tr>
<th></th>
<th>Living kidney</th>
<th>Brain Dead kidney</th>
<th>Non-Heart-Beating kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (U/min/gram)</td>
<td>0.133±0.029</td>
<td>0.098±0.034</td>
<td>0.222±0.022*</td>
</tr>
<tr>
<td>NAG (U/min/gram)</td>
<td>1.8±0.5</td>
<td>0.5±0.1</td>
<td>6.9±1.1*</td>
</tr>
</tbody>
</table>

*P<0.05 vs. living

Table 3. Biochemical parameters, determined in urine, reflecting renal injury in living and brain dead kidneys after 90 min isolated kidney perfusion

<table>
<thead>
<tr>
<th></th>
<th>Living kidney</th>
<th>Brain Dead kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAG (µU/min/ml/gram)</td>
<td>0.25±0.05</td>
<td>0.09±0.01*</td>
</tr>
<tr>
<td>AAP (µU/min/ml/gram)</td>
<td>0.29±0.05</td>
<td>0.13±0.03*</td>
</tr>
<tr>
<td>AP in urine (µU/min/ml/gram)</td>
<td>3.16±0.54</td>
<td>1.34±0.24*</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>3.90±1.01</td>
<td>4.97±1.14</td>
</tr>
</tbody>
</table>

*P<0.05 vs. living

Enzyme histochemistry of alkaline phosphatase (AP)
Non-specific AP immunoreactivity and enzyme activity were observed in the proximal neprhon of all groups. In Figure 3, the extent of damage in NHB, brain dead, and living donor kidneys is illustrated. AP was particularly stained in the brush border membrane cells of proximal tubules. A high intensity of AP staining was than also found in proximal tubular cells located in the cortex. Interstitium, glomeruli, and blood vessels were negative for AP. In contrast to the living group, medullary structures (tubular cells, collecting tubules) and the lumen in especially the proximal tubular cells were focally positive for AP in both the brain dead and NHB group, however, to a much

Figure 2. Fractional K+ reabsorption in living and brain dead kidneys during isolated kidney perfusion. L-donor: living donor. BD-donor: brain dead donor. Values are mean ± SEM. *P<0.01, **P<0.001.
larger extent in the NHB group (Figure 3A, 3C, and 3E). A similar pattern was found for alanine amino peptidase (data not shown). Cells of the nephron, particularly proximal tubular cells, were disrupted during brain death and cardiac arrest. Reperfusion disrupted cells of the nephron, since AP was not only stained in the cortex (proximal and distal tubular cells), but also in medullary structures (tubular cells, collecting tubules) of perfused living, brain dead, and NHB kidneys (Figure 3B, 3D, and 3F).

Figure 3. Microscopic analysis of enzyme histochemical staining of alkaline phosphatase (AP) in living, brain dead, and non-heart-beating kidneys before (A, C, and E) and after isolated kidney perfusion (B, D, and F).
Discussion

In the present study we have examined the effect of brain death and of cardiac arrest with warm ischemia (non-heart-beating (NHB) donor) on donor kidney function and injury. To determine these effects, the isolated perfused kidney (IPK) model was used. Kidneys from brain dead and NHB donor rats showed significant differences in intrarenal vascular resistance (IRR), urine production, glomerular filtration rate (GFR), K+ reabsorption, and glucose metabolism compared to living donor kidneys. Also, major differences amongst these groups in release of brush border, lysosomal enzymes, as well as intracellular enzymes were seen.

An increase of IRR during ischemia is associated with a decrease in donor kidney viability. In our study, NHB kidneys showed increased IRR compared to kidneys from the living group. After 90 min of IPK, the IRR, however, decreased to a value similar to the IRR found in the living and brain dead kidneys. Previously, in human and animal studies, it has been reported that IRR is increased in brain dead and NHB kidneys [17-19]. The pathogenesis of IRR in the ischemia-related injury of the kidney, as in the NHB donor, is complex. IRR reflects the constrictive response of the renal vascular wall to the ischemic insult. Although the exact mechanism of increase of IRR is not clear yet, it has been postulated that local release of vasoconstrictive agents (endothelium-derived relaxing factor, i.e. nitric oxide) and swelling of damaged endothelial cells may contribute to the process of IRR increase [20-23].

Kidneys in the brain dead group produced more urine during IPK than those derived from living donor rats. The water channel protein aquaporin-2 is involved in water reabsorption in the renal collecting ducts. It is regulated in its expression and activity by the antidiuretic hormone vasopressin probably via the V2-receptor. Sudden increase in urine volume with a decrease in urine osmolarity ("vasopressin escape") is coincided with a decrease in aquaporin-2 protein and mRNA expression as well as a decrease in V2-receptor mRNA expression and binding [24]. This may very well occur during brain death, since diabetes insipidus, accompanied by high urinary output and decrease in urine osmolarity, is developed, and serum vasopressin concentrations are significantly decreased in brain dead patients [25-27]. Also, aquaporin-2 expression or activity could be disturbed in the kidneys derived from brain dead rats. This may explain the increase in urine production by the brain dead group during IPK. Recently, we found in brain death experiments with Wistar rats (HSD.Cpb:WU) [2,3], that induction of brain death resulted in the downregulation of aquaporin-2 mRNA expression (unpublished results).

GFR was significantly lower in NHB kidneys compared to kidneys from the living group. In this study, the clearance of inulin was used as a measurement for GFR. Since clearance is dependent on the amount of urine production, a deterioration of GFR in NHB kidneys can be explained by a reduced urinary output. Two out of six NHB kidneys did not produce urine at all and four kidneys produced very little urine (71±43 µl/min/gram) during IPK. This was significantly less compared to kidneys from the living group (312±64 µl/min/gram). Urine production, and, thereby, GFR of brain dead kidneys was almost twice as good (588±65 µl/min/gram) than that of living kidneys (Table 1).

Oliguria or anuria in the early period after transplantation are characteristic for NHB donor kidneys [6,8]. The reason for this phenomenon is still unclear, but might be caused by acute tubular necrosis induced during ischemia [28]. The increased incidence of primary non and never-function (PNF) and delayed graft function (DGF) in clinical kidney transplantation is the most common complication when NHB donor kidneys are used. DGF occurs in 40-80% and PNF in 4-14% of patients that have been transplanted with a NHB donor kidney [6-10]. From PNF and DGF, DGF is the most pertinent variable in the outcome of poor graft function, since most of the studies show a comparable incidence of PNF between the NHB and heart-beating group [7-9].

Reabsorption of Na+ and K+ reflects tubular function. Whereas Na+ reabsorption could be determined in four out of six NHB kidneys, K+ reabsorption could not be determined, due to their insufficient urinary output. NHB kidney donation did not effect Na+ reabsorption. In contrast to NHB and Na+ reabsorption, the brain dead kidneys showed a net excretion of K+ (negative K+ reabsorption) during IPK, indicating a continuous loss of K+ in the urine. In the clinical situation, increased urine K+ levels have been found in brain dead patients. These patients require constant infusion of potassium, to replace urinary K+ loss [29]. Na+ and K+ homeostasis is regulated by the Na+-K+-ATPase pump and ATP-sensitive potassium (K<sub>ATP</sub>) channels.
Both are present in proximal tubular cells, medullary thick ascending limbs, and cortical collecting ducts. The Na\(^+\)-K\(^+\)-ATPase pump is involved in the release of Na\(^+\) and the uptake of K\(^+\). K\(_{ATP}\) channels are involved in the release of K\(^+\). A decrease in intracellular ATP level results in the opening of K\(_{ATP}\) channels and, thereby, excretion of K\(^+\) [30]. These channels are activated by the rapid fall of ATP levels during renal ischemia, resulting in cellular injury, which occurs due to the leak of cellular K\(^+\) [31]. Occurrence of ATP depletion in 24-hr machine perfused stored brain dead hearts has been reported [32]. The amount of ATP in non-stored brain dead kidneys was 0.78±0.04 pmol/µg protein, and was significantly lower than in living kidneys (1.48±0.23 pmol/µg protein). Thus, ATP depletion did already occur in non-stored brain dead kidneys, which may lead to enhanced K\(^+\) leakage. Leakage of K\(^+\) from cells disturbs the ionic balance and contributes to decreased activity of the Na\(^+\)-K\(^+\)-ATPase pump. Although, Na\(^+\) reabsorption is also regulated by the Na\(^+\)-K\(^+\)-ATPase pump, Na\(^+\) reabsorption was found to be not affected by brain death in this study. This can be explained by involvement of other mechanisms in Na\(^+\) reabsorption that are less sensitive for ischemia, e.g. the Na\(^+\)-H\(^+\) pump and / or reabsorption across the paracellular pathway [33].

Other relevant data for evaluating renal function and cellular damage after transplantation were glucose metabolism and lactate dehydrogenase (LDH) release. Glucose can be metabolized either aerobically or anaerobically. Aerobic metabolism of glucose results in CO\(_2\) and H\(_2\)O and a high ATP yield. Anaerobic metabolism of glucose results in lactate and a low ATP yield. Glucose metabolism is, therefore, an indicator of the functional activity of an organ. Based on the measured lactate levels in the perfusate of kidneys from NHB rats, an increased anaerobic metabolism could be observed, compared to living and brain dead rats. Although, brain dead kidneys showed aerobic metabolism of glucose, the findings on the ATP amount in these kidneys, as just discussed, suggest that they probably produce ATP in an inefficient way. On the other hand, lactate concentration in the perfusate of NHB kidneys was higher, resulting in significantly more anaerobic metabolism glucose than in the kidneys from the living group. Also, LDH concentration in the perfusate of brain dead kidneys was not significantly increased, whereas significantly higher LDH levels were found in the perfusate of NHB kidneys compared to kidneys from living rats. LDH release is markedly increased when kidneys were retained under ischemic conditions during IPK [34]. In a canine autotransplantation model, it has been shown that increase of LDH release is associated with the duration of warm ischemia of the kidney [4]. Therefore, the long warm ischemia time in NHB donation leads to increased cellular damage.

Effects of ischemia on renal injury have been histologically and biochemically studied. Renal ischemic injury is accompanied by disruption of the cytoskeleton of proximal tubular cells and the loss of brush border [35]. Intracellular and membrane-bound enzymes, such as alanine aminopeptidase (AAP) and alkaline phosphatase (AP) are located in the brush border. In addition, lysosomal enzymes, such as N-acetyl-β-D-glucosaminidase (NAG), are present in the nephron. Urinary concentration of these enzymes is increased after ischemic events [36,37]. Enzyme histology of AP showed injury in the nephron, particularly in proximal tubular cells, and loss of brush border in the kidneys from brain dead and NHB rats (Figure 3). In contrast to normal kidneys from living rats, AP and AAP were both found in the medulla (tubular cells, collecting tubules) and in the lumen of tubular cells of brain dead and NHB kidneys. Non-IPK and IPK kidney sections showed that the extent of nephron injury was high in the NHB, moderate in the brain dead, and low in the living group. Nephron injury during IPK is a characteristic feature and may depend on the amount of oxygen that can be consumed [38,39]. In the living and brain dead group, injury of the nephron and brush border during IPK was reflected in the secretion of AAP, AP, and NAG in the urine. In the NHB group, NAG was significantly increased in the perfusate. Due to the low urine production, enzyme activity could not be measured in the NHB group. Enzyme activity was significantly lower in the urine of the brain dead group compared to the living group. Possibly the secretion of brush border and lysosomal enzymes in the urine during the brain death period is underlying the lower enzyme release during IPK. Also, the data concerning NAG activity in the perfusate suggest that in kidneys, e.g. NHB kidneys, showing PNF or DGF the lysosomal enzyme NAG can be extracellularly secreted by nephron segments.

In this study, we showed functional differences between injured (brain death, NHB) and non-injured kidneys (living). Kidneys from NHB donor rats were
deteriorate in function of urine production. This is in agreement with the clinical situation of PNF and DGF [6,8]. Urine production in general is higher than normal in the kidneys from brain dead rats. This might be due to the reduction of hormones (a.o. vasopressin), which are involved in the regulation of water homeostasis, as has been described for brain dead patients [25-27]. A net excretion of K⁺ (negative K⁺ reabsorption) was observed in brain dead donor kidneys, suggesting loss of K⁺ in the urine, as has been reported for brain dead patients [29]. Disruption of K<sub>ATP</sub> channels through ATP depletion during brain death, previously shown in a porcine brain death model using machine perfused stored hearts [32] and suggested in the present study in a rat brain death model using non-stored kidneys, might be a possible explanation. The finding of enzyme activity of the brush border enzymes AAP and AP, and of the lysosomal enzyme NAG in the urine and perfusate of living, brain dead and NHB kidneys, respectively, might be useful markers in the prediction of the incidence of PNF and/or DGF.

In summary, brain death and cardiac arrest with its inevitable long warm ischemia time have different effects on renal function, but did not differ much in extent of renal injury. The NHB kidneys showed pronounced functional damage in the IPK set-up compared to brain dead kidneys. The brain dead donor may therefore be preferable to the NHB donor based on functional analysis. On the other hand, both brain dead and NHB kidneys show tubular injury and are inferior in quality compared to the living donor kidneys. Furthermore, the brain dead kidney has been shown to be immunologically activated that cannot be studied in an IPK model.

References:

24. Ecelbarger CA, Murase T, Tian Y, Nielsen S, Neppe Motor V, Verbalis JG. Regulation of Renal Salt and Water Transporters During
Vasopressin Escape. Prog. Brain Res. 2002;139:75-84.


