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Renal transplantation induces mitochondrial uncoupling, increased kidney oxygen consumption, and decreased kidney oxygen tension

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Renal transplantation induces mitochondrial uncoupling, increased kidney oxygen consumption, and decreased kidney oxygen tension. Am J Physiol Renal Physiol 308: F22–F28, 2015. —Hypoxia is an acknowledged pathway to renal injury and ischemia-reperfusion (I/R) and is known to reduce renal oxygen tension (PO2). We hypothesized that renal I/R increases oxidative damage and induces mitochondrial uncoupling, resulting in increased oxygen consumption and hence kidney hypoxia. Lewis rats underwent syngenic renal transplantation (TX) and contralateral nephrectomy. Controls were uninephrectomized (1K-CON) or left untreated (2K-CON). After 7 days, urinary excretion of protein and thiobarbituric acid-reactive substances were measured, and after 14 days glomerular filtration rate (GFR), renal blood flow, whole kidney QO2, cortical PO2, kidney cortex mitochondrial uncoupling, renal oxidative damage, and tubulointerstitial injury were assessed. TX, compared with 1K-CON, resulted in mitochondrial uncoupling mediated via uncoupling protein-2 (16 ± 3 vs. 0.9 ± 0.3 pmol O2·s·mg protein−1·min, P < 0.05) and increased whole kidney QO2 (55 ± 16 vs. 33 ± 10 µmol O2·min·P < 0.05). Corticomedullary PO2 was lower in TX compared with 1K-CON (30 ± 13 vs. 47 ± 4 µM, P < 0.05) whereas no significant difference was observed between 2K-CON and 1K-CON rats. Proteinuria, oxidative damage, and the tubulointerstitial injury score were not significantly different in 1K-CON and TX. Treatment of donors for 5 days with mito-TEMPO reduced mitochondrial uncoupling but did not affect renal hemodynamics, QO2, PO2, or injury. Collectively, our results demonstrate increased mitochondrial uncoupling as an early event after experimental renal transplantation associated with increased oxygen consumption and kidney hypoxia in the absence of increases in markers of damage.

mitochondrial uncoupling; oxidative damage; transplantation; hypoxia

KIDNEY TRANSPLANTATION (TX) is the definitive treatment for end-stage renal disease. Even though most transplantations are successful, with a graft survival rate of 90% within 1 yr, delayed graft function (DGF) and interstitial fibrosis/tubular atrophy (IF/TA, previously denoted as chronic allograft nephropathy) (47) remain serious clinical problems (30, 43, 44). Ischemia-reperfusion (I/R) is an inevitable event accompanying kidney TX and is considered a common cause for DGF and acute renal failure, ultimately resulting in IF/TA (3, 5, 7, 25). Mechanisms leading to DGF and IF/TA after renal TX are poorly understood, and, at present, we lack therapies to prevent I/R injury.

I/R is accompanied by an increased mitochondrial production of reactive oxygen species (ROS) (37), an event that is likely to induce mitochondrial uncoupling. Uncoupling proteins (UCPs) can be directly activated by superoxide radicals (12) to release protons independently of ATP production, thereby lowering the membrane potential and decreasing superoxide production. Indeed, mitochondrial uncoupling has been shown to occur in the kidneys of hypertensive and diabetic animal models (6, 8, 16, 36).

In most tissues, mitochondrial uncoupling functions as an antioxidant mechanism. However, increased mitochondrial uncoupling is always accompanied by increased oxygen consumption to sustain ATP production, a side effect that is potentially detrimental for the kidney. Increased renal blood flow (RBF) does not correct the increase in oxygen consumption, as increased RBF will inevitably result in increased glomerular filtration rate (GFR) and therefore an increased tubular load of electrolytes destined for active reabsorption. Thus increased oxygen delivery is matched by increased demand. The inability of the kidney to compensate for increases in oxygen consumption renders it particularly sensitive to alterations in oxygen metabolism that result in decreased kidney oxygen tension (PO2). In 1998, it was proposed by Fine et al. (15) that the kidney has a hypoxic threshold that, when overstepped, initiates mechanisms that cause nephropathy. Interestingly, a lower renal PO2 is observed in rats with hypertension (49), diabetes (32), and after I/R (31), and chronic kidney hypoxia is now an acknowledged pathway to end-stage renal disease (26, 28, 29).

Increased mitochondrial fragmentation and production of ROS and oxidative damage have been shown to occur after I/R (4, 37, 48), but studies have yet to show the presence of kidney mitochondrial uncoupling or describe its connection to kidney oxygenation, function, and oxidative damage. We hypothesize that increased mitochondrial uncoupling and the associated increase in kidney oxygen consumption lead to kidney hypoxia early after experimental renal TX and that this increase precedes damage. Our aim was therefore to investigate mitochondrial function, in vivo kidney function, oxygen metabolism, and markers of oxidative and structural damage in transplanted kidneys compared with one-kidney controls. In a follow-up study, we evaluated whether the scavenging of mitochondrial superoxide in the donor before the TX procedure could improve these variables.

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METHODS

Rats

Male inbred Lewis rats (LEW/Crl), 300–350 g, were purchased from Charles River and housed in a climate-controlled facility with a 12:12-h light-dark cycle under standard conditions. All rats had free access to standard rat chow and tap water. The study protocol was approved by the Utrecht University Committee on Animal Experiments, conformed to Dutch Law on Laboratory Animal Experiments (DEC number 2010.II.05.097) and the Uppsala Animal Ethics Committee [Ethics Statement (c143/12)], and was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Design

All chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Study 1. Age-matched rats of 8–10 wk were used as 2-kidney controls (2K-CON; n = 13), uninephrectomized rats as 1-kidney controls (1K-CON; n = 12), or underwent syngeneic renal TX (TX; n = 12). Seven days after surgery, rats were placed in metabolic cages to collect 24-h urine for evaluation of excretion of protein (Bio-Rad Protein assay, Bradford, Bio-Rad, Hercules, CA) and thiobarbituric acid-reactive substances (TBARS; TBARS assay kit, Cayman Chemical, Ann Arbor, MI). Fourteen days after surgery, mitochondrial uncoupling and renal function, including arterial, venous, and direct tissue PO2, were measured in separate cohorts. Directly after measurements of renal function, rats were euthanized and renal tissue was fixed in 4% paraformaldehyde for embedding in paraffin, or snap-frozen to measure protein carbonyl content in the kidney cortex. Protein carbonyls were determined with a kit (Cayman Chemical).

Study 2. Donors were treated intravenously with mito-TEMPO, a mitochondrial-targeted antioxidant, a specific scavenger of mitochondrial superoxide (200 μg/kg, TX-T, n = 11; Enzo Life Sciences, Farmingdale, NY) or vehicle (0.9% NaCl, TX-V, n = 10) daily for 5 days before TX. mito-TEMPO (100 μM) was added to an organ-preserving Viaspan solution (Bristol-Myers Squibb) during cold storage of the kidneys from the TX-T group. All measurements of oxidative damage, mitochondrial uncoupling, and renal function and injury were determined as for study 1.

Transplantation Procedure

Renal TX was performed as described (46). Briefly, via laparotomy, the left kidney of the donor rat was flushed with 0.9% NaCl followed by a flush with the Viaspan solution. Afterward, the donor kidney was kept in Viaspan on ice for 30 min (cold ischemia time).
TX was performed with end-to-end anastomoses of vessels and the urer, with 30- to 40-min warm ischemia time and subsequent removal of the contralateral kidney. Thus survival of the recipient was dependent on the function of the renal graft.

Mitochondrial Uncoupling

Mitochondria were isolated from kidney cortex as described (36). Oxygen consumption in isolated mitochondria was measured with an Oroboros O2K (Oroboros Instruments, Innsbruck, Austria) with 10 mM glutamate (state 4) and 300 μM ADP (state 3), and the respiratory control ratio (RCR) was determined as state 3/state 4 respiration. Only mitochondria with an RCR >4 were used for experiments (18). Mitochondrial uncoupling was evaluated in isolated mitochondria in the presence of glutamate (donates electrons to the electron transport chain), oligomycin (12 μg/mg protein; an ATP synthesis inhibitor), and sodium palmitate (48 μM; a fatty acid to enable the fatty acid cycling mechanism of UCP). This level of oxygen consumption is denoted as state 4 respiration, will be evident as an increased state 4 respiration. If an increased state 4 respiration is mediated through UCPs, the sequential addition of the UCP-specific inhibitor GDP (500 μM) will inhibit oxygen consumption. The presence of mitochondrial uncoupling is calculated and presented as the decrease in oxygen consumption that occurs after UCP blockade. If no mitochondrial uncoupling mechanism is present, oxygen consumption will not be affected by GDP and the value presented will be close to zero. Mitochondrial membrane potential was determined by fluorescence of tetramethylrhodamine methyl ester (TMRM) as described (18). Before the isolation process, some kidney cortical tissue was snap-frozen for analysis of protein carbonyls. All these measurements were corrected for protein concentration.

In Vivo Renal Function

Renal function was investigated under isoflurane anesthesia (Abbot Laboratories, Hoofddorp, The Netherlands) as described (24). Renal function data are shown per kidney, and one-kidney function in the 2K-CON group was calculated as 50% of total renal function. At the end of the experimental period, blood-gas analysis was performed on arterial blood and on a sample obtained from the renal vein. Sampling from the renal vein was performed slowly to prevent backflow of blood from the vena cava. Blood oxygen content (O2ct) was calculated as O2ct = [hemoglobin] * oxygen saturation * 1.34 + blood PO2 * 0.003, and kidney oxygen consumption was calculated as QO2 = (O2ct artery – O2ct renal vein) * total RBF. Carbon paste electrodes placed at 3-mm depth using a micromanipulator were used to determine PO2 at the corticomedullary border (mean of at least 3 samplings/rat) (23). In a pilot experiment, after measurement of kidney PO2 with a carbon paste electrode, the kidneys were cut through the plane where the electrode was inserted. With a ruler we confirmed that this depth indeed corresponds to the corticomedullary border, as shown previously (34). Plasma urea and plasma creatinine were determined by DiaSys Urea CT FS (DiaSys Diagnostic Systems, Holzheim, Germany). Sodium and potassium were measured by flame photometry. Fractional excretions of sodium and potassium (FeNa and FeK) were calculated using standard formulas. Tubulointerstitial injury (TI) was scored on periodic acid Schiff (PAS)-stained paraffin-embedded slides (22).

Statistics

Data were analyzed with one-way ANOVA with Dunnett’s test as a post hoc test using 1K-CON for comparison with both 2K-CON and TX (study 1) or an unpaired Student’s t-test for comparison of TX-T and TX-V (study 2). P < 0.05 was considered statistically significant and was two-tailed. All values are expressed as means ± SD.

RESULTS

Kidney TX Led to Mitochondrial Uncoupling, Increased Oxygen Consumption, and Decreased Total Kidney Oxygenation (Study 1)

Seven days after surgery, excretion of TBARS and protein in urine was greater in 1K-CON rats compared with 2K-CON rats (Fig. 1A and Table 1). At 14 days after surgery, protein carbonyls from kidney cortex were increased in 1K-CON vs. 2K-CON (Fig. 1B) rats. TBARS and protein carbonyls were not significantly different in TX vs. 1K-CON groups. Mitochondria isolated from kidneys of TX rats displayed higher mitochondrial uncoupling via UCP-2 vs. kidneys of 1K-CON rats (Fig. 1D), which was associated with an elevated membrane potential after UCP-2 blockade by GDP (Fig. 1C). No significant differences were found in RCR in isolated mitochondria (Table 2). In TX and 1K-CON rats, no significant difference was found in urea, creatinine, GFR, RPF, and RBF. Urea, hematocrit, GFR/kidney, RPF/kidney, and RBF/kidney were lower in 2K-CON compared with 1K-CON rats. No significant differences were found in MAP (Table 1). Kidney oxygen consumption was higher

Table 1. Clinical signs, renal function, and histology in age-matched healthy controls (2K-CON) and 2 wk after uninephrectomy (1K-CON) or renal transplantation (TX)

<table>
<thead>
<tr>
<th></th>
<th>2K-CON (n = 10)</th>
<th>1K-CON (n = 7)</th>
<th>TX (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>398 ± 42</td>
<td>346 ± 35</td>
<td>367 ± 25</td>
</tr>
<tr>
<td>Total renal mass, μg/100 g body weight</td>
<td>701 ± 44†</td>
<td>509 ± 51</td>
<td>545 ± 55</td>
</tr>
<tr>
<td>Plasma urea, mmol/l</td>
<td>7.7 ± 1.2*</td>
<td>9.5 ± 1.5</td>
<td>9.6 ± 1.6</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>27.2 ± 6.3</td>
<td>37.5 ± 5.5</td>
<td>37.7 ± 3.8</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43.7 ± 1.2†</td>
<td>40.0 ± 1.4</td>
<td>38.2 ± 1.5†</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>96 ± 6</td>
<td>97 ± 10</td>
<td>99 ± 11</td>
</tr>
<tr>
<td>Glomerular filtration rate per kidney, ml/min⁻¹·100 g⁻¹</td>
<td>0.47 ± 0.05†</td>
<td>0.61 ± 0.09</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>Renal plasma flow per kidney, ml/min⁻¹·100 g⁻¹</td>
<td>1.82 ± 0.22†</td>
<td>2.45 ± 0.44</td>
<td>2.58 ± 0.23</td>
</tr>
<tr>
<td>Calculated renal blood flow per kidney, ml/min⁻¹·100 g⁻¹</td>
<td>2.9 ± 0.3†</td>
<td>3.9 ± 0.7</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Fractional excretion Na, %</td>
<td>0.30 ± 0.20</td>
<td>0.17 ± 0.08</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>Fractional excretion K, %</td>
<td>37.5 ± 6.3</td>
<td>44.1 ± 5.7</td>
<td>37.9 ± 6.8</td>
</tr>
</tbody>
</table>

|                      | 2K-CON (n = 13) | 1K-CON (n = 13) | TX (n = 13)  |
| Proteinuria at day 7, mg·day⁻¹·100 g⁻¹·kidney⁻¹ | 1.0 ± 0.5†      | 2.4 ± 0.5       | 2.1 ± 1.5    |
| Tubulointerstitial injury score | 1.8 ± 0.9*      | 2.6 ± 0.8       | 2.7 ± 0.8    |

Values are means ± SD. *P < 0.05, †P < 0.01 vs. 1K-CON (1-way ANOVA, Dunnett’s post hoc test).

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and kidney PO2 was lower in TX compared with 1K-CON rats (Fig. 1, E and F). In comparison to 1K-CON, 2K-CON rats displayed less tubulointerstitial injury (Table 1). Tubulointerstitial injury was not significantly different in TX vs. 1K-CON rats.

**Donor Pretreatment with Mito-TEMPO Decreased Mitochondrial Uncoupling But Did Not Affect Oxygen Consumption or Total Oxygenation (Study 2)**

Pretreatment of donor rats with mito-TEMPO and addition of mito-TEMPO to the organ-preserving solution did not affect excretion of TBARS in urine at day 7 (Fig. 2A) or protein carbonyls in tissue at day 14 (Fig. 2B). The degree of mitochondrial uncoupling via UCP-2 was lower compared with mitochondria isolated from vehicle-treated rats (Fig. 2C), and mitochondrial membrane potential after GDP was higher compared with baseline in both vehicle- and mito-TEMPO-treated rats (Fig. 2D), but no significant differences were observed in kidney oxygen consumption (Fig. 2E) or kidney PO2 (Fig. 2F). RCR of isolated mitochondria was not affected (Table 2). Parameters of renal function and injury were not affected (Table 3).

![Table 2. Mitochondrial oxygen consumption during state 4 and state 3 respiration and the calculated respiratory control ratio (RCR)](table2.jpg)

Values are means ± SD. *P < 0.05 vs. 1K-CON (1-way ANOVA, Dunnett’s post hoc test).

![Fig. 2. Excretion of TBARS in urine at day 7 (A), protein carbonyl content in the kidney cortex (B), mitochondrial uncoupling (C), mitochondrial membrane potential (D), kidney oxygen consumption (E), and kidney oxygen tension (F) in vehicle (TX-V)- and mito-TEMPO (TX-T)-treated rats that underwent kidney TX. B–F: 14 days after TX. Values are means ± SD. *P < 0.01 vs. TX-V (A–C, E, and F) and vs. baseline (D); unpaired Student’s t-test.](fig2.jpg)
Table 3. Clinical signs, renal function, and histology 2 wk after transplantation with daily vehicle (TX-V) or mito-TEMPO (TX-T) donor treatment for 5 days before donation for transplantation

<table>
<thead>
<tr>
<th></th>
<th>TX-V (n = 6)</th>
<th>TX-T (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>341 ± 19</td>
<td>325 ± 12</td>
</tr>
<tr>
<td>Total renal mass, µg/100 g body wt</td>
<td>564 ± 53</td>
<td>584 ± 71</td>
</tr>
<tr>
<td>Plasma urea, mmol/l</td>
<td>33.9 ± 11.6</td>
<td>33.6 ± 3.3</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40 ± 2</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>88 ± 7</td>
<td>96 ± 9</td>
</tr>
<tr>
<td>Glomerular filtration rate per kidney, ml-min⁻¹-100 g⁻¹</td>
<td>0.62 ± 0.04</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>Renal plasma flow per kidney, ml-min⁻¹-100 g⁻¹</td>
<td>0.22 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Calculated renal blood flow per kidney, ml-min⁻¹-100 g⁻¹</td>
<td>12.1 ± 1.7</td>
<td>12.6 ± 0.9</td>
</tr>
<tr>
<td>Fractional excretion Na, %</td>
<td>0.11 ± 0.09</td>
<td>0.15 ± 0.13</td>
</tr>
<tr>
<td>Fractional excretion K, %</td>
<td>45.8 ± 7.3</td>
<td>40.1 ± 8.3</td>
</tr>
<tr>
<td>Proteinuria at day 7, mg·day⁻¹-100 g⁻¹·kidney⁻¹</td>
<td>2.3 ± 0.9</td>
<td>2.6 ± 1.1</td>
</tr>
<tr>
<td>Tubulointerstitial injury score</td>
<td>3.4 ± 1.4</td>
<td>3.2 ± 0.8</td>
</tr>
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</table>

Values are means ± SD.

DISCUSSION

The present study shows that experimental renal TX induces mitochondrial uncoupling mediated via UCP-2. Furthermore, renal TX was accompanied by increased kidney oxygen consumption and decreased kidney PO₂. Interestingly, in other models of animals without underlying disease but with increased oxygen consumption due to mitochondrial uncoupling (from either pharmacological stimuli or hormonal stimuli), increased kidney oxygen consumption and decreased kidney PO₂ were also observed (19, 20). Kidney hypoxia is now an acknowledged pathway in the development of nephropathy (26). However, in the present study we found no effect of TX on markers of oxidative damage or injury in the TX kidney compared with relevant 1K-CON, suggesting that the changes in renal oxygen handling induced by TX in the present study precede renal injury. Therefore, we speculated that mitochondrial uncoupling as an early event after renal TX may be a mechanism contributing to renal graft injury and DGF. However, it should be noted that the present study does not allow for definitive conclusions on causality.

Using blood oxygen level-dependent (BOLD)-MRI, several groups have displayed increased kidney oxygenation in renal allografts. No difference was observed in patients with normal allograft function compared with controls (35), while decreased deoxyhemoglobin levels that correspond to increased oxygenation were reported in patients with IF/TA (9). This effect could be caused by decreased oxygen consumption that is observed in patients after inflammatory and immune-mediated kidney damage (10). Impaired renal function with decreased GFR would also reduce the workload of electrolyte transport for the kidney and therefore contribute to a higher PO₂ in these patients. Interestingly, the BOLD-MRI-studies (10) contradict the observations of the present study of slightly decreased renal oxygenation after TX. However, these studies were performed once IF/TA was established. Importantly, the present study measured renal oxygenation 14 days after TX and will therefore reflect the early effects of renal TX that are likely to contribute to a later loss of kidney function and reduced workload, ultimately resulting in increased oxygenation. In addition, to avoid the confounding effects of immunosuppression we used a syngenic model of TX to specifically study the effect of I/R after TX. To our knowledge, we are the first to study the mechanism of altered oxygen handling as an early event after renal TX.

There are also other mechanisms besides mitochondrial uncoupling that could have influenced kidney PO₂ in the 1K-CON and TX groups in the present study. Kidney PO₂ represents the balance between oxygen delivery (represented by RBF) and oxygen consumption, but it can also be influenced by the arterial-to-venous (A-V) oxygen shunting in the renal cortex and medulla (14). Studies have suggested that A-V shunting increases with increased RBF, and, indeed, increased RBF was observed after TX. However, RBF was similarly increased in 1K-CON, making increased shunting due to increased RBF an unlikely cause of decreased kidney PO₂ after TX. Moreover, there were no clear differences in gross morphology (evaluated as TI injury) between these two groups.

Studies have demonstrated that UCPs can directly be activated by superoxide radicals (11, 12), and oxidative damage is known to induce mitochondrial uncoupling in the kidneys of diabetic animals (36). As mitochondrial production of ROS is also increased after I/R (37), we hypothesized that mitochondrial uncoupling occurs after experimental renal TX. Indeed, we found an increase in mitochondrial uncoupling after experimental renal TX that was associated with increased levels of oxidative damage. The level of mitochondrial uncoupling that we observed was dependent on GDP, an inhibitor of UCPs. As UCP-2 is the only isoform of UCP present in the kidney (17), it can be assumed that the observed mitochondrial uncoupling was mediated via UCP-2. Interestingly, mitochondrial membrane potential was not different between groups without any incubation but was increased after UCP blockade in kidneys from TX rats. Thus in our TX model mitochondrial uncoupling does not decrease membrane potential below normal levels but only uncouples the membrane potential back to control levels, maintaining mitochondrial production of superoxide at physiological levels. In concordance with this, we observed no signs of oxidative damage at 7 and 14 days after TX vs. 1K-CON.

Studies have demonstrated a connection between oxidative stress and mitochondrial uncoupling (32, 36), and it was therefore interesting to note that treating the donors with the mitochondrial-targeted antioxidant mito-TEMPO resulted in reduced mitochondrial uncoupling compared with vehicle-treated TX rats. However, mito-TXPO had no effects on renal function and oxygen handling. Mitochondria-targeted antioxidants are antioxidant molecules connected to tetraphenylphosphonium, a lipophilic cation that accumulates in the mitochondria due to the negative membrane potential. These molecules have been shown to be effective in the treatment of various pathologies such as acute pyelonephritis (38), hypertension (8), and cardiac I/R injury (1). In the present approach, in which only donors are treated, it is evident that the degree of mitochondrial superoxide scavenging is not sufficient to completely prevent mitochondrial uncoupling. Mito-TEMPO did reduce the level of mitochondrial uncoupling. However, levels of oxidative damage markers were not different between vehicle- and mito-TEMPO-treated TX rats. The failure of mito-
TEMPO to scavenge all ROS could occur because the level of oxidative stress may continue to increase for some time after TX. Indeed, even the remaining degree of mitochondrial uncoupling was greater than what was previously observed in kidneys from untreated diabetic animals (16, 18, 36), and it is possible that the increased degree of mitochondrial uncoupling reflects the severity of insult and subsequent levels of oxidative stress. It is likely that for decreased mitochondrial uncoupling to be protective it must be completely prevented, as demonstrated in diabetic kidneys (36). The concept of preventing mitochondrial uncoupling after experimental TX using antioxidants is an interesting approach for future studies. Although mitochondrial uncoupling was reduced after mito-TEMPO, the mitochondrial membrane potential was similar in mitochondria from both groups after UCP inhibition. Importantly, there is not a linear relationship between uncoupling and membrane potential change in mitochondria (27). Thus the relatively small effect of mito-TEMPO on mitochondrial uncoupling may not be associated with a measurable change in mitochondrial membrane potential.

Kidney $PO_2$ was decreased after TX together with increased oxygen consumption. Although no cause-effect relationship can be established in this study, it is important to note that previous studies have shown that increased kidney oxygen consumption indeed results in kidney hypoxia and development of nephropathy (19, 20). Decreased kidney $PO_2$ has been observed in rat models of hypertension (49, 50), diabetes (32, 39, 40), polycystic kidney disease (2), and after I/R injury (18, 37). Furthermore, decreased renal oxygenation was also observed with BOLD-MRI in patients with diabetes and chronic kidney disease (21). Other human studies also support a role for kidney hypoxia in the development of kidney damage. Diabetic patients living at 1,700 m above sea level have increased prevalence of diabetic nephropathy compared with similar patients living at sea level, but these to two groups were not different in terms of mean arterial pressure, glycemia, or lipidemia status or prevalence of retinopathy (42). Furthermore, an observational study correlated the degree of nocturnal hypoxemia with accelerated decline in GFR (41). In summary, kidney hypoxia is now regarded as an independent causal pathway in the development of nephropathy (6, 8, 12, 13, 33, 45). The present study did not reveal a connection between hypoxia and development of nephropathy, possibly due to the early time point of analysis. We speculate that decreased $PO_2$ soon after experimental renal TX, as observed in the present study, could play a long-term role in DGF and the development of IF/TA.

Conclusion and Future Perspectives

This is the first study to demonstrate UCP-2-mediated mitochondrial uncoupling accompanied by increased kidney oxygen consumption and decreased kidney $PO_2$ as an early event after experimental renal TX. These early events may contribute to development of DGF and IF/TA after kidney TX.

Future studies should focus on investigating the role of UCP-2 mediated mitochondrial uncoupling and kidney hypoxia in the long-term outcome of experimental renal TX, the putative role of oxidative stress in inducing mitochondrial uncoupling, and how the damaging effects of mitochondrial uncoupling can be prevented.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.A.P., M.F.-P., J.A.J., and M.C.V. conception and design of research; D.A.P. and M.F.-P. performed experiments; D.A.P., M.F.-P., and J.A.J. analyzed data; D.A.P., M.F.-P., J.A.J., and M.C.V. interpreted results of experiments; D.A.P. and M.F.-P. prepared figures; D.A.P. and M.F.-P. drafted manuscript; D.A.P., M.F.-P., J.A.J., and M.C.V. edited and revised manuscript; D.A.P., M.F.-P., J.A.J., and M.C.V. approved final version of manuscript.

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