Objectives: Acute kidney injury is a serious complication with unacceptably high mortality that lacks specific curative treatment. Therapies focusing on the hydraulic behavior have shown promising results in preventing structural and functional renal impairment, but the underlying mechanisms remain understudied. Our goal is to assess the effects of renal decapsulation on regional hemodynamics, oxygenation, and perfusion in an ischemic acute kidney injury experimental model.

Methods: In piglets, intra renal pressure, renal tissue oxygen pressure, and dysoxia markers were measured in an ischemia-reperfusion group with intact kidney, an ischemia-reperfusion group where the kidney capsule was removed, and in a sham group.

Results: Decapsulated kidneys displayed an effective reduction of intra renal pressure, an increment of renal tissue oxygen pressure, and a better performance in the regional delivery, consumption, and extraction of oxygen after reperfusion, resulting in a marked attenuation of acute kidney injury progression due to reduced structural damage and improved renal function.

Conclusions: Our results strongly suggest that renal decapsulation prevents the onset of an intrinsic renal compartment syndrome after ischemic acute kidney injury.

Key Words: acute kidney injury; intrarenal pressure; ischemia reperfusion; renal decapsulation; renal tissue oxygen pressure
shown promising results in ameliorating AKI progression. Studies in a murine model of transplantation have shown that reducing the intrarenal pressure (IRP) prevents functional and structural renal impairment (12). Further, in patients with AKI associated with abdominal compartment syndrome, an increment of RPP due to the release of intra-abdominal pressure has effectively reduced renal injury (13, 14). Such findings suggest that a therapeutic approach that considers the hydraulic behavior of the kidney has important clinical implications in AKI management (15). In a previous contribution, we studied the dependence of IRP on the intrarenal fluid volume in porcine kidneys with intact kidney capsule. A highly nonlinear relation between the IRP and the injected volume was found, confirming the existence of a mechanical behavior similar to that observed in other organs confined by a rigid or semirigid structure, which we referred to as the “intrinsic renal compartment syndrome.” In contrast, kidneys whose capsule was removed presented a linear pressure-volume relation with IRP levels always below those found for the intact kidney case, confirming the role of the renal capsule as a confiner (16). From a translational perspective, and in analogy to other compartment syndromes, renal decapsulation and its potential increase in RPP due to a reduction in IRP may have important implications in the prevention of a secondary injury in AKI (15). Although the beneficial hydraulic outcomes of renal decapsulation have been addressed in the literature (12, 16), its therapeutic benefits have been understudied to date.

In this work, we hypothesize that renal decapsulation prevents the development of an intrinsic renal compartment syndrome, attenuating structural and functional renal impairment. Our main goal is to elucidate the effects of renal decapsulation on the renal hemodynamics, regional tissue oxygenation and perfusion, as well as on structural damage and function, in a porcine model of ischemic AKI.

METHODS

Animal Preparation

Large white piglets (11.9 ± 1.0 kg) were provided by a local vivarium. The experimental protocol was approved by the institutional review board of the Universidade André Bello and followed the Guiding Principles in the Care and Use of Laboratory Animals of the American Physiological Society. The study was powered to detect a 30% reduction in IRP. The sample size per group needed to achieve an 80% study power was six, with a 0.05 two-sided significance level.

Animals were premedicated with intramuscular midazolam (0.5 mg/kg), methadone (0.5 mg/kg), and ketamine (15 mg/kg), followed by induction with intravenous propofol (3 mg/kg). Tracheal intubation was performed with auffed tracheal tube (5 mm internal diameter; Mallinckrodt Shiley, St. Louis, MO), followed by inhalation anesthesia with isoflurane 1.5%. After local infiltration with 1% lidocaine, each animal’s left jugular vein was cannulated via cut down with a 7F triple lumen catheter (Arrow, Reading, PA) and the right femoral artery with a 4F thermistor-tipped catheter (PiCCO PV2014L08; Pulsion Medical Systems, Munich, Germany). Body temperature was continuously monitored throughout the duration of the experiments. A forced-air warming device and insulating surgical drapes were employed to maintain a target body temperature of 38°C ± 0.5°C. Anesthesia and neuromuscular blockade were maintained by continuous infusion of propofol (10 mg/kg/hr), fentanyl (5 μg/kg/hr), and vecuronium (0.3 mg/kg/hr) throughout the experiment. Animals were initially ventilated with a Fabius GS ventilator and later with an EVITA 4 ventilator (both from Dräger Medical, Lübeck, Germany) using the volume-control mode. Initial settings were tidal volume equals to 10 mL/kg, positive end-expiratory pressure equals to 5 cm H2O, Fio2 equals to 0.4, inspiratory time equals to 1.0 second, and respiratory rate (RR) equals to 20 breaths/min. RR was adjusted to achieve a Paco2 of 40 ± 10 torr.

After surgical preparation, block randomization was used to assign piglets to the ischemia-reperfusion (I/R) group, the decapsulated I/R (D-I/R) group, and the sham group, with a total of six animals per group. All animals were placed in supine decubitus position, and a midline laparotomy was performed. Both kidneys were identified and dissected free of surrounding tissue. All animals were allowed a 30-minute stabilization period before baseline data were collected. Immediately after stabilization, the renal arteries and veins in the I/R and D-I/R groups were cross-clamped for 45 minutes, after which the clamp was released and the kidneys were observed for immediate reperfusion. In the D-I/R group, renal decapsulation was performed immediately after clamping, following the technique described by Stone and Fülenwinder (17). The capsule was first incised and elevated at its lateral margin, then cut from the superior to the inferior pole and finally stripped apart in the medial plane. The abdomens of all animals were closed by a contained laparotomy, and the piglets were monitored for 240 minutes. A timeline schematic of the experimental protocol is provided in Supplementary Figure 1 (Supplemental Digital Content 1, http://links.lww.com/CCM/C981; legend, Supplemental Digital Content 2, http://links.lww.com/CCM/C981).

Measurements

After laparotomy, hemodynamic, respiratory, and renal measurements were recorded at the baseline, during the clamping, and 240 minutes after reperfusion. Pao2, Paco2, and hemoglobin were assessed using i-STAT cartridges (Abbott Laboratories, Princeton, NJ) with blood samples drawn from the arterial catheter. Heart rate, mean arterial pressure (MAP), and central venous pressure (CVP) were monitored during the experiment (Infinity Delta XL; Dräger Medical, Lübeck, Germany). Zero pressure was set at the midaxillary line. Cardiac output, global end-diastolic volume (GEDV), and extravascular lung water (EVLW) were evaluated in triplicate by means of transpulmonary thermodilution using a commercially available device (PiCCO; Pulsion Medical Systems, Munich, Germany). The body surface area of each piglet, expressed in m2, was calculated as K/weight (in kilograms)0.23, where K equals 0.112 for pigs (18). IRP was measured using a catheter Camino 4B inserted 1 cm inside the left lower renal pole and connected to Camino Single Parameter Monitor Model.
Renal tissue oxygen pressure (PtiO₂) was measured using a Clark-type polarographic catheter Licox IMC IT2 inserted 0.5 cm inside the left lower renal pole and connected to a Licox CMP monitor (Integra NeuroSciences). PtiO₂ was recorded after 20 minutes of stabilization. Renal blood flow (RBF) was measured using ecodoppler (MyLab25Gold; Esaote, Genova, Italy). At the end of the study, the left renal vein was cannulated with a 22G BD Angiocath catheter (Becton Dickinson, Franklin Lakes, NJ) to measure the renal venous pressure (RVP). Renal vascular resistance (RVR) was computed as (MAP – RVP)/RBF.

Arterial and renal venous lactate was amperometrically measured using i-STAT Cartridges CG4+ (Abbott Laboratories). Organ-specific lactate production was computed as the product of blood flow in the organ and the difference between efferent and afferent lactate concentrations. Using Fick’s equation, renal lactate release (RLR) was calculated as the difference between the L-lactate renal venous concentration and the L-lactate arterial concentration, multiplied by RBF, and expressed as mmol/min/m². Renal venous hemoglobin oxygen saturation (SRVO₂) was assessed in blood samples from the renal vein catheter using i-STAT cartridges (Abbott Laboratories). Renal oxygen delivery (DO₂-R = RBF × 1.34 × hemoglobin × arterial oxygen saturation [SaO₂]), renal oxygen uptake (VO₂-R = RBF × 1.34 × hemoglobin × [SaO₂ – SrO₂]), and renal oxygen extraction (EO₂-R = VO₂-R/DO₂-R) were calculated. Urine samples were collected using a Cistofix catheter (B. Braun Medical, Melsungen, Germany) directly connected to the bladder. Urinary output was volumetrically measured at intervals of 60 minutes and reported in ml/kg/hr. Serum creatinine level was measured using the enzyme method, whereas urinary albumin levels were measured by an immunoturbidimetric assay from the urine sample collected during the last hour of the study. Serum neutrophil gelatinase-associated lipocalin (NGAL) measurements were performed using enzyme-linked immunosassays (Lipocalin2/NGAL Pig ELISA Kit; Abcam, Cambridge, MA).

Anesthetized animals were euthanized by administration of 10% potassium chloride injection until the detection of ventricular fibrillation or asystole. The left kidney was removed, cleaned of connective tissue, and renal tissue samples were fixed in 10% formalin, dehydrated in ethanol, embedded in paraffin, and cut into 4-μm thick slices. Then, the sections were deparaffinized with xylene and stained with hematoxylin and eosin. Tissue samples were acquired from the renal caudal pole in all subjects. A blinded evaluation of histopathology slides was performed by an experienced pathologist. Results were expressed in terms of a histologic score, as defined in Bernet et al (19). The samples were mounted to detect in situ apoptosis using the apoptotic cells ApopTag Peroxidase In Situ Apoptosis Detection Kit (Merck Millipore Corporation, Darmstadt, Germany). Apoptosis in tissue was assessed using an apoptotic index, defined as the apoptotic nuclear area × 100/total nuclear area, where nuclear area was determined from computer-assisted morphometry analysis (20) (Image Pro-Plus; Media Cybernetics, Rockville, MD) in five random locations within each tissue sample.

**Statistical Analysis**

Data are expressed as mean ± SEM. Normality was assessed by the Anderson-Darling test. Comparisons between groups were made using the Mann-Whitney one-tailed U test. Bonferroni correction was applied to account for the multiple comparison analysis, which for comparisons between all three groups gives a Bonferroni coefficient of 3. The correlation between variables was determined using the Spearman test. Significance was set at p value of less than 0.05 (one-sided). All statistical analyses were performed using the Python programming language and the statistical package of SciPy 0.17.0 (Python Software Foundation, http://www.python.org).

**RESULTS**

All animals completed the experimental protocol. Before induction of renal ischemia, no significant differences in hemodynamic, respiratory, and renal variables were found between the groups under study (Supplementary Table 1, Supplemental Digital Content 2, http://links.lww.com/CCM/C981).

Renal perfusion and oxygenation (Fig. 1): At the end of the study, the IRP in the I/R group with intact capsule was significantly higher than the IRP in both the D-I/R group and the sham group. For the PtiO₂, the I/R group resulted in significantly lower values than both the D-I/R and sham groups. No differences in the RPP, RVR, and RBF were found between all experimental groups, but MAP was higher in the I/R group than in the sham group.

Renal dysxia (Fig. 2): RLR was found to be greater in the I/R group than in the D-I/R group. SrO₂ in the I/R group was greater than both the D-I/R and sham groups. DO₂-R in the I/R group was smaller than in the D-I/R group. Both the V₀₂-R and EO₂-R were significantly smaller in the I/R group than in the D-I/R group and sham group.

Renal dysfunction (Fig. 3): Both I/R and D-I/R groups resulted in smaller levels of urine output and greater levels of urinary albumin/creatinine ratio and NGAL than the sham group. Serum creatinine was greater in the I/R group than the sham group.

Histopathologic evaluation (Fig. 4): No histologic alterations were observed in the kidneys of Sham piglets. The histologic score shows that, while both I/R and D-I/R groups suffered damage, only the structural damage in the I/R kidneys was significantly more severe than the damage found in the sham’s kidneys. All three comparisons between groups showed significantly different apoptotic indices, with the I/R group having the highest value and the sham the lowest value.

Extrarenal crosstalk (Supplementary Fig. 2, Supplemental Digital Content 3, http://links.lww.com/CCM/C982; legend, Supplemental Digital Content 2, http://links.lww.com/CCM/C981): No significant differences in Pao₂, Paco₂, and CVP were found between experimental groups (not shown). No differences between groups were observed for GEDV. EVLW was greater in the I/R group than in the sham group.

A negative correlation was found between IRP and PtiO₂ (Fig. 5A). Positive correlation coefficient values were obtained for the relation between IRP and NGAL, as well as for IRP and...
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Histologic score (Fig. 5, B and C, respectively). Ptio2 exhibited a negative correlation with NGAL and with histologic score (Fig. 5, D and E, respectively).

**DISCUSSION**

In this work, we show that renal decapsulation following an ischemic AKI effectively reduces the IRP. Previous work in an ischemic AKI murine model also showed that IRP reduction was possible after capsule removal. However, the mechanisms that prevented severe structural and functional renal impairment were unclear (12). Our study of renal tissue oxygenation confirms and extends these results by showing that renal decapsulation results in an effective increase in Ptio2. Such increase in renal tissue oxygenation may be explained by the increase of RBF in the D-I/R group when compared with the I/R group, and not necessarily due to an increase in RPP. Despite decapsulation results in lower IRP than that observed in AKI kidneys with intact capsule, it does not have a significant effect on the RPP.

![Graph showing various parameters](image_url)

**Figure 1.** Renal perfusion and oxygenation in the experimental groups at the end of the study. All data are presented as means ± SEM. *p < 0.05, **p < 0.01. Bonferroni correction (m = 3) is used for multiple comparisons. D-I/R = ischemia-reperfusion plus decapsulation group, I/R = ischemia-reperfusion group, IRP = intra renal pressure, MAP = mean arterial pressure, Ptio2 = renal tissue oxygen pressure, RBF = renal blood flow, RPP = renal perfusion pressure, RVR = renal vascular resistance.

![Graph showing various parameters](image_url)

**Figure 2.** Markers of renal dysoxia in the experimental groups at the end of the study. All data are presented as means ± SEM. *p < 0.05, **p < 0.01. Bonferroni correction (m = 3) is used for multiple comparisons. D-I/R = ischemia-reperfusion plus decapsulation group, DO2-R = renal oxygen delivery, EO2-R = renal oxygen extraction, I/R = ischemia-reperfusion group, RLR = renal lactate release, SvenO2 = renal venous hemoglobin oxygen saturation, VO2-R = renal oxygen uptake.
Figure 3. Markers of renal dysfunction in the experimental groups at the end of the study. All data are presented as means ± SEM. *p < 0.05, **p < 0.01. Bonferroni correction (m = 3) is used for multiple comparisons. ACR = albumin/creatinine ratio, D-I/R = ischemia-reperfusion plus decapsulation group, I/R = ischemia-reperfusion group, NGAL = serum neutrophil gelatinase-associated lipocalin.

Figure 4. Representative histopathological images for the ischemia-reperfusion (I/R) group, the decapsulated I/R (D-I/R) group, and the Sham group. 

A. Renal decapsulation prevents structural damage after ischemic acute kidney injury. In reference to the healthy kidney (c, f, i), kidneys subjected to 45 min of ischemia exhibit extensive tissue damage. Massive and intense degenerative changes including picnosis, cariorrexis, and cariolysis due to cell death affected the epithelium of cortical tubules with most tubules showing large amounts of protein and cell debris in dilated lumen (a, d). Large cortical areas show intense staining for DNA fragmentation associated to apoptosis (g). Kidneys exposed to prolonged ischemia but treated by renal decapsulation present well-preserved renal morphology and excellent viability with no signs for necrosis (b, e) and reduced apoptosis (h). Hematoxylin-eosin stain. 

B. Histologic score. 

C. Apoptotic index. Bonferroni correction (m = 3) is used for multiple comparisons. TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling stain.
In contrast to ischemic AKI kidneys with intact capsule, we show that decapsulated kidneys perform markedly better at the delivery, consumption, and extraction of oxygen, along with a decreased RLR. Although these findings are novel for the case of the kidney, they have been studied and reported in decompressive craniectomy (DC) in patients with refractory intracranial hypertension (21–25). Recent studies have shown that DC not only results in a reduction of the intracranial pressure but also in an increment in tissue oxygenation, as well as in reduced levels of regional dysoxia markers. These are considered to be more direct indicators of an improved balance between oxygen consumption and delivery and are associated to improved clinical outcome (22, 25, 26). In our case, we associate higher levels of PtiO₂ in decapsulated kidneys not only to the increase of Do₂-R but also to a tissue oxygen consumption similar to that observed in the sham group. In contrast, we show that pathologically high levels of SRVO₂ are found in the intact capsule ischemic AKI kidney. The combination of low levels of V̇O₂-R with an elevated production of lactate strongly suggests anaerobic metabolism in the renal parenchymal tissue, which has important implications in limiting the progression of AKI (27, 28).

Our histologic analyses confirm that renal decapsulation drastically reduces the structural damage in ischemic AKI, a finding suggested by previous studies (12). Although both ischemic AKI groups show a significant reduction of renal function, decapsulation effectively attenuates functional impairment (Fig. 3B). Interestingly, we observe a marked trend that indicates that structural and functional damage increases in severity as the level of IRP increases and the level of PtiO₂ decreases, as revealed by our correlation study (Fig. 5). This suggests that AKI severity is modulated both by IRP and PtiO₂, which are correlated but are not necessarily dependent.

Renal dysfunction is known to play a role in the development of distant organ failure (29, 30). Here, we show that decapsulation attenuates pulmonary edema, as revealed by EVLW (Supplementary Fig. 2, Supplemental Digital Content 3, http://links.lww.com/CCM/C982; legend, Supplemental Digital Content 2, http://links.lww.com/CCM/C981). These findings suggest that renal decapsulation may have important implications in distant organ dysfunction (31, 32). The underlying mechanisms associated to the extrarenal crosstalk in ischemic AKI should be further elucidated by measuring inflammatory pathways, for example, cytokine levels in the blood stream.

Our study suffers from some limitations. First, decapsulation was performed shortly after inducing ischemic AKI, which differs from the clinical setting where therapeutic interventions are initiated several hours, or even days, after the initial insult. Early renal decapsulation after vascular clamping can have important clinical implications in preventing secondary injury in kidney transplantation. Indeed, the reduction of renal dysfunction and structural damage in decapsulated kidneys undergoing I/R may play a key role in the prevention of graft dysfunction (12).

Second, the duration of our experimental model is limited to a few hours; thus, it can only give insights into the short-term effects of renal decapsulation. Despite such a short time span, the attenuation of structural damage and renal dysfunction in the decapsulated group is remarkable, particularly
when compared with the poor outcome of AKI kidneys with intact capsule, highlighting the potential harm of a renal intrinsic compartmental syndrome which can be developed in a few hours. Third, the duration of induced ischemia was fixed in all of our experiments and did not consider shorter ischemic periods. As a consequence, the beneficial effects of decapsulation reported in this study are associated to kidneys undergoing long periods of ischemia and may change in the case of shorter ischemic periods. Finally, it is worth remarking that we have not studied pathophysiologic mechanisms such as mitochondrial function and microcirculation. Future studies should focus on such cellular mechanisms to understand both how a compartmental syndrome worsens AKI and how decapsulation attenuates the progression of AKI.

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