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Physiologic homeostasis after pig-to-human kidney xenotransplantation

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Abstract

Demand for kidney grafts outpaces supply, limiting kidney transplantation as a treatment for kidney failure. Xenotransplantation has the potential to make kidney transplantation available to many more patients with kidney failure, but the ability of xenografts to support human physiologic homeostasis has not been established. A brain-dead adult decedent underwent bilateral native nephrectomies followed by 10 gene-edited (four gene knockouts, six human transgenes) pig-to-human xenotransplantation. Physiologic parameters and laboratory values were measured for seven days in a critical care setting. Data collection aimed to assess homeostasis by measuring components of the renin-angiotensin-aldosterone system, parathyroid hormone signaling, glomerular filtration rate, and markers of salt and water balance. Mean arterial blood pressure was maintained above 60 mmHg throughout. Pig kidneys secreted renin (post-operative day three to seven mean and standard deviation: 47.3 ± 9 pg/mL). Aldosterone and angiotensin II levels were present (post-operative day three to seven, 57.0 ± 8 pg/mL and 5.4 ± 4.3 pg/mL, respectively) despite plasma renin activity under 0.6 ng/mL/hr. Parathyroid hormone levels followed ionized calcium. Urine output down trended from 37 L to 6 L per day with 4.5 L of

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DISCLOSURE

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electrolyte free water loss on post-operative day six. Aquaporin 2 channels were detected in the apical surface of principal cells, supporting pig kidney response to human vasopressin. Serum creatinine down trended to 0.9 mg/dL by day seven. Glomerular filtration rate ranged 90-240 mL/min by creatinine clearance and single-dose inulin clearance. Thus, in a human decedent model, xenotransplantation of 10 gene-edited pig kidneys provided physiologic balance for seven days. Hence, our in-human study paves the way for future clinical study of pig-to-human kidney xenotransplantation in living persons.

Keywords

aldosterone; parathyroid hormone; renin-angiotensin system; transplantation; water channels

More than 800 million people worldwide have chronic kidney disease, and >1 million die each year of kidney failure.[1,2] In the United States, kidney disease is the 10th leading cause of death and responsible for $87 billion in Medicare-related spending, with renal replacement therapy accounting for most of this expenditure.[3] These statistics are staggering given the gold standard treatment of kidney transplantation was developed 69 years ago. Demand for kidney grafts outpaces supply, and this limits kidney transplantation as a treatment option to few individuals with kidney failure. Radical solutions, like xenotransplantation, provide a means to close the gap between kidney supply and demand.

The largest barrier to implementation of xenotransplantation has been xenograft rejection. Recent advancements in genetic editing tools have allowed the generation of humanized pig kidneys for purposes of human transplantation. Early but promising results from preclinical trials in human decedents have moved xenotransplantation into the realm of reality. Most recently, we performed pig-to-human kidney xenotransplantation in a brain-dead research decedent and demonstrated, for the first time, life-sustaining kidney function in an anephric human, as evidenced by production of urine and clearance of creatinine.[4] Although encouraging, these results shed light on only a fraction of post-xenotransplant pig kidney physiology that will be critical to assess and understand before moving into trials in living persons.

Kidney physiology is complex, encompassing the clearance of metabolic waste products from the blood, maintenance of fluid-electrolyte and acid-base balance, hormonal signaling affecting red blood cell production, and bone health. Thus, the kidneys are contributors to overall homeostasis and the primary regulators of blood pressure. Whether porcine kidneys are capable of regulating this complex physiology in humans is unknown. Although xenotransplantation studies in pig-to-nonhuman primate (NHP) models have suggested that porcine kidneys are sufficiently similar to human kidneys to support the physiological needs of a living person, adult humans are physiologically distinct; NHPs are smaller in size and have lower mean arterial pressures. As such, NHP models may be insufficient to assess the ability of porcine kidney xenografts to support the needs of living persons with kidney disease.

Pig kidneys have fewer total nephrons and fewer long-looped nephrons compared with human kidneys.[5] Moreover, pig vasopressin is more potent than human vasopressin,

suggesting human vasopressin may be less potent on porcine kidney receptors. Porcine renin is unable to efficiently cleave human angiotensinogen, suggesting the renin-angiotensin-aldosterone system (RAAS) may be impaired after kidney xenotransplantation.[6,7] Knowledge gaps exist with regard to the ability of porcine kidneys to metabolize circulating hormones, such as insulin, or respond to parathyroid hormone (PTH). These differences are not trivial and pose the risk of physiological derangement after xenotransplant, including hypovolemia, hypotension, and electrolyte abnormalities. The ability to understand and quantify pig kidney xenograft physiology in a human model is critical to the safe execution of pig-to-human kidney xenotransplant phase 1 clinical trials. Herein, we address this substantial knowledge gap and describe, for the first time, postxenotransplant porcine kidney physiology in a human.

METHODS

Study decedents

In this case series, as previously described, adult (aged ≥18 years) brain-dead persons (decedents) whose families provided informed consent for study participation were eligible for study entry after all organ donation options were exhausted.[8] The study was approved by the University of Alabama at Birmingham Institutional Review Board (number 300004648). The study followed the Appropriate Use and Reporting of Uncontrolled Case Series in the Medical Literature reporting guideline.

Porcine kidney donors

Pigs with 10 genetic edits, including 4 gene knockouts (*GTKO, CMAH, B4GALNT2,* and *GHR*) and 6 human transgenes (*CD46, CD55, CD47, THBD, PROCR,* and *HMOX1*), were maintained in a pathogen-free facility and negative for porcine endogenous retrovirus C, porcine cytomegalovirus, and other zoonoses (Supplementary Table S1). Porcine donors received general anesthesia for procurement and were humanely euthanized thereafter.[8] The 10 gene-edited porcine kidneys were flushed with the University of Wisconsin solution, sterile packaged, cold stored on ice, and transported via ground to University of Alabama at Birmingham for xenotransplantation. The study was approved by the Institutional Animal Care and Use Committee (number 22015).

Pig-to-human xenotransplantation—Tissue compatibility was assessed using flow crossmatch, as previously described.[8] Bilateral native nephrectomies were performed through a midline incision. Both porcine donor kidneys were transplanted en bloc to the decedent's right common iliac artery and distal inferior vena cava. Porcine donor kidney ureters were anastomosed to the decedent's bladder. Xenotransplantation was performed in an operating theater meeting Joint Commission on Accreditation of Healthcare Organizations standards.

Immunosuppression

On the basis of results from previous studies in NHPs, a complement inhibitor (anti-C5, eculizumab) was administered i.v. 24 hours before (1200 mg) and 24 hours after (900 mg) xenotransplantation. Induction immunosuppression included i.v. methylprednisolone (500

mg), anti-thymocyte globulin (6 mg/kg total given in divided doses [1.5 mg/kg] over 4 days), and anti-CD20, rituximab (375 mg/m^2). The i.v. methylprednisolone was tapered over 4 days for immunosuppression. Additional corticosteroid dosing was administered throughout the experiment to manage brain death. Maintenance immunosuppression included calcineurin inhibitor (tacrolimus; goal level, 8–12 ng/dl), mycophenolate mofetil (1000 mg twice daily), and prednisone (30 mg once daily). Tacrolimus, mycophenolic acid, and complement levels were followed daily. In addition, a tacrolimus pharmacokinetic study was performed. Specifically, tacrolimus levels (ng/ml) were drawn at time of administration on postoperative day 4 and then 2, 4, 6, 8, 10, and 12 hours after administration; an area under the curve was then calculated.

Assessment of RAAS

Levels of renin, angiotensinogen, angiotensin II, and aldosterone were measured following the protocol used in prior xenotransplant experiments in NHPs.[9]

PTH signaling

Serum PTH levels, ionized calcium, phosphorus, creatinine, total 25-OH vitamin D, and 1,25 DI-OH vitamin D, along with random urine concentrations of phosphorus and creatinine, were measured daily. The fractional excretion of phosphorus was calculated.

Measures of kidney clearance (endogenous and exogenous)

Kidney clearance was assessed by measuring 24-hour flow, serum inulin clearance, creatinine clearance, and cystatin C-based glomerular filtration rate (GFR) estimation. Blood samples for sodium, chloride, creatinine, and cystatin C, and random urine samples for sodium, chloride, and creatinine were obtained daily. Following a validation study of GFR measurement in NHPs, inulin clearance was measured by analyzing the decay curve after bolus dosing[10] on postoperative day 5 after serum creatinine nadir and stabilization (Supplementary Figure S1). Serum inulin was measured by enzyme-linked immunosorbent assay (BioPAL; number FIT-0416). The area under the inulin serum concentration versus time curve was calculated. Inulin total body clearance after the bolus dose was calculated using the following formula:

$$Clearance_{IV\ bolus} = \frac{Dose}{Area\ under\ the\ curve_{0-infinity}}$$

Masures of kidney salt and water handling

Higher blood glucose levels from glucocorticoid use in the treatment of brain death resulted in glucosuria. To account for the osmotic effect of urinary glucose, electrolyte-free water clearance was calculated as a measure of kidney water handling. Electrolyte-free water clearance (L) was calculated using the following formula:

$$Clearance_{water} = 24hr\ Urine\ Volume * \left(1 - \frac{Urine_{Na} + Urine_{K}}{Serum_{Na}}\right)$$

Daily measured urine osmolality and dipstick urinalyses provided specific gravity and glucose levels. Anti-diuretic hormone levels were measured by send-out hospital laboratory intermittently, and serum copeptin was measured before the inulin clearance by an enzyme-linked immunosorbent assay (Cloud Corp.; number CEA365Hu).

Measures of collecting duct function

Aquaporins (AQPs) were detected using immunohistochemistry methods previously reported.[11] Primary antibodies were AQP1 (Proteintech; 1:2500 dilution), AQP2 (Santa Cruz Biotechnology; clone E2; 1:1000), AQP2-phosphorylated S256 (Abcam; 1:1000), and AQP4 (Abcam; 1:2000). Primary antibodies were diluted in 2.5% normal horse serum (Vector Labs) and on the tissues for 24 hours at 4 °C. Primary antibody binding was detected using ready-to-use secondary antibodies (Vector Labs) and ImmPACT DAB Peroxidase Substrate (Vector Labs). Additional slides were used to immunolocalize AQP2 (principal cells) and vacuolar-type H+–adenosine triphosphatase B1/2 (intercalated cells) using immunofluorescent techniques, as previously reported.[11] The anti–vacuolar-type H+–adenosine triphosphatase B1/2 was 1:100 (Santa Cruz Biotechnology; sc-55544) diluted in 2.5% normal horse serum placed on the tissue for 1 hour and then detected with 1:1000 goat–anti-mouse 595. This was followed by incubation with 488-directly tagged AQP2 (clone E2; 1:100). Nuclei were visualized with 4′,6-diamidino-2-phenylindole. Negative controls lacking primary antibodies were included (Supplementary Figure S2). Images were taken with an Olympus Bx53 microscope and DP28 digital camera. Urine pH was measured using serial dipstick urinalyses.

Measurement of proteinuria

Serial 24-hour urine collections allowed for quantification of albumin and total protein in the urine.

RESULTS

Demographics

A 53-year-old man with a history of hypertension, type 2 diabetes, and acute kidney injury superimposed on chronic kidney disease stage 2 was enrolled. Tissue compatibility was confirmed by negative flow crossmatch (Supplementary Figure S3). Continuous renal replacement therapy was stopped, and bilateral native nephrectomies were performed. Serum creatinine peaked at 3.9 mg/dl after cessation of dialysis and bilateral native nephrectomy. Pig-to-human kidney xenotransplantation was then performed, and physiological performance was Measures of kidney assessed over the 7-day study period. The porcine donor was a 14-month-old, 92.2-kg, male, 10 gene-edited pig with normal kidney function (Supplementary Table S1).

RAAS, vasopressin, copeptin, and electrolyte levels

Mean arterial pressure remained >60 mm Hg throughout the study period (Supplementary Figure S4). Notably, the decedent was not hypotensive after xenotransplantation and required a nicardipine drip to treat hypertension. Plasma renin concentrations ranged from 37.1 to 61.3 pg/ml after xenotransplantation (Figure 1a). However, plasma renin activity

was below the level of detection (<0.6 ng/ml per hour) throughout the study duration. Plasma angiotensinogen levels ranged from 92 μg/ml on postoperative day 1 to 58.7 μg/ml on postoperative day 7, comparable to healthy humans (Figure 1b).[12] Plasma angiotensin II increased from 0.6 pg/ml on postoperative day 4 to 10.6 pg/ml on postoperative day 7 (Figure 1c).[13] Plasma aldosterone levels were low, ranging from 65 pg/ml on postoperative day 1 to 44.2 pg/ml on postoperative day 7 (Figure 1d). Serum potassium concentrations remained between 3.1 and 4.6 mEq/L throughout the study duration, with a reduction in i.v. potassium supplementation from 160 to 200 mEq/d on postoperative days 1 to 4 to 40 mEq/d on day 6 (Supplementary Table S2). Serum magnesium levels were maintained near 1.9 mg/dl with minimal infusion support (Supplementary Table S2). Serum vasopressin concentrations averaged 6.4 pg/ml, and copeptin was 0.26 pg/L on postoperative day 5.

PTH axis

PTH level was elevated before xenotransplantation and increased to 1015 pg/ml on postoperative day 1, with a corresponding ionized calcium level <1.0 mmol/L. With i.v. supplementation (Supplementary Table S3), ionized calcium increased to >1 mmol/L on postoperative day 2 and remained stable through study duration. In parallel, PTH decreased to 455.9 pg/ml on postoperative day 2 and remained between 232.1 and 386.1 pg/ml through study duration (Figure 2). The decedent was found to be vitamin D deficient before xenotransplantation, with total 25-OH vitamin D level of 7 ng/ml. Serum phosphate levels remained between 4.2 and 7.4 mg/dl after xenotransplantation; however, urinary phosphate excretion remained normal, with a fractional excretion of phosphate averaging 29% in the last 3 days of the study.

Clearance

GFR increased in the first 5 days after xenotransplantation, to a peak of 240.7 ml/min by 24-hour urine creatinine clearance on postoperative day 4 and 231.6 ml/min by inulin clearance on postoperative day 5 (Figure 3a and b). In the afternoon of postoperative day 5, decline in urine flow due to urinary obstruction from a kinked Foley catheter was discovered and corrected. The GFR returned to 150 ml/min by 24-hour urine creatinine clearance on postoperative day 7 (Figure 3b). The trend in GFR changes mirrored trends in serum creatinine levels (Figure 3b and c). Estimated GFR by cystatin C measurement remained lower than other methods for assessing GFR (Figure 3b). Pharmacokinetic studies demonstrated tacrolimus trough levels ranging from 8 to 10 ng/dl, with an area under curve 0–12 hours ($AUC_{0\text{-}12}$) of 103.34 ng*h/ml (Figure 3d).

Salt and water handling

Urine output was 37 L in the first 24 hours of xenotransplantation (Figure 4a). Serum sodium levels increased sharply, peaking at 167 mEq/L on postoperative day 2 (Figure 4b). As per standard brain death management protocol at Legacy of Hope, the decedent was on a low-dose continuous vasopressin infusion to replace pituitary function. In response to increasing serum sodium levels, replacement fluid was switched to one-half normal saline, and a total of 2 doses of desmopressin were administered i.v. (2 and 1 μg on postoperative days 2 and 3, respectively) with gradual decline of serum sodium levels to the normal range on postoperative day 3. Serum osmolality levels ranged from 285 to 312 mOsm/kg

and were often >300 mOsm/kg, representing high blood glucose levels. The presence of glucosuria (urinalyses detected glucose at 1+ to 2+ on all 7 postoperative days) kept urine osmolality high despite net water loss (Figure 4c and d). Urinary water loss peaked on postoperative day 3 at 9.5 L/d and stabilized on postoperative days 5 to 7 between 3 and 4.5 L/d (Figure 4c). Urine osmolality was 230 mOsm/kg on postoperative day 1 and peaked at 429 mOsm/kg by day 6 (Figure 4d). Hemodynamic support with i.v. fluid offset urinary losses. The i.v. fluid administration decreased from 16 to 7 L/d throughout the study (Supplementary Table S4).

Collecting duct function

AQPs were immunolocalized in the pig kidney. AQP1 was abundant in the apical membrane of the proximal tubules (Figure 5a). The basolateral membrane of the principal cells of the collecting duct had detectable AQP4 (Figure 5b), whereas the apical/subapical membrane expressed AQP2 (Figure 5c). Moreover, phosphorylation of AQP2-S256, an indicator of active AQP2, was also present in the principal cells (Figure 5d). Vacuolar-type H+–adenosine triphosphatase B1/2 levels were localized on the apical surface of intercalated cells (Figure 5e and Supplementary Figure S2), and the urine pH remained low at 5 for the last 5 days of the experiment. Trichrome-stained kidney sections of the cortex (Figure 5f) and medulla (Figure 5g) showed no obvious pathology.

Proteinuria

Protein levels in the urine were initially nephrotic range at 8.9 g of total protein and 3.5 g of albumin on postoperative day 1. By postoperative day 6, 24-hour total protein had reduced to 3.24 g, with 0.95 g of albumin.

DISCUSSION

For the first time, we have established the ability of a 10-gene edited porcine kidney xenograft to maintain physiological homeostasis in a human. The porcine xenograft cleared both endogenous and exogenous substrates, including the most common maintenance immunosuppressant used in transplantation, provided sufficient RAAS activity to maintain normal hemodynamics and avoid hyperkalemia, sufficiently concentrated urine to make daily enteral water intake feasible, secreted acid, and demonstrated appropriate hormonal response to hypocalcemia. Understanding the physiological underpinnings of pig-to-human kidney xenotransplantation is critical to ensuring the safety and feasibility of porcine kidney xenografts as a treatment option for persons with end-stage kidney failure.

Limited studies in the genetic edited pig-to-NHP models indicate porcine kidney xenografts can maintain normal serum creatinine but have provided few details regarding clearance of endogenous and exogenous substrates. Renal clearance as a metric is pivotal to understanding the ability of a kidney graft (xenograft or allograft) to provide immediate and long-term life-sustaining kidney function. Previously, in the preclinical human Parsons model, we have demonstrated life-sustaining kidney function after 10 gene-edited porcine kidney xenotransplantation as measured by the clearance of creatinine in the absence of native kidneys or dialysis support.[4] In the current report, we build on those findings with

evidence of exogenous clearance of inulin, as well as pharmacokinetic study of tacrolimus area under the curve, consistent with findings after human-to-human allotransplantation. Moreover, we demonstrated that the endogenous clearance marker, cystatin C, was not a reliable marker of GFR estimation in pig-to-human kidney xenotransplantation. Our findings suggest that porcine kidney xenografts will perform renal clearance of exogenous and endogenous substrates similar to human kidney allografts.

The pig kidney has a reduced ability to concentrate urine and retain water compared with human kidneys given data from the pig-to-NHP xenotransplant model, where urine osmolality levels remained <400 mOsm/kg despite intermittent hypotension.[9] The likelihood of impaired urinary concentrating ability of porcine kidney xenografts due to species-specific differences between human arginine vasopressin and pig lysine vasopressin is a substantial knowledge gap in our understanding of pig kidney physiology and could have significant consequences for human xenograft recipients. Before the present study, the ability of pig kidneys to concentrate urine had never been tested in a human recipient. Consistent with observations from NHP models, we observed voluminous urine output initially after xenotransplantation with corresponding hypernatremia. Over the course of the 7-day study period, the urine output decreased and serum sodium was normalized. We calculated urinary water losses between 3 and 4.5 L/d, and given the GFR, this means 99% of the filtered water was reabsorbed. AQP expression in the proximal tubules and principal cells of the collecting duct was abundant and found in expected subcellular compartments; AQP1 was abundant in the proximal tubules, AQP2 was in the apical membrane, and AQP4 was in the basolateral membrane of the collecting duct principal cells.[14-16] AQP2 is vasopressin responsive, and vasopressin results in increased trafficking of AQP2 to the apical membrane to drive water reabsorption.[17] This is mediated through the phosphorylation of serine 256 in the C-terminus of AQP2, and AQP2-S526 was detected in the apical membrane of the principal cells of the pig kidney.[18] Thus, the localization of these water channels was normal and consistent with the water reabsorption reported. In the brain-dead model, a vasopressin infusion is required to replace reduced hypothalamic-pituitary function. Low levels of copeptin (<1 pg/L) on postoperative day 5 confirmed little endogenous vasopressin release. With an intact pig-kidney response to human vasopressin, water losses from anatomic limitations in urine concentration could be managed by thirst and water intake. High volume of urine output, up to 20 L, is common in the first day after a living kidney transplant with intraoperative diuretic dosing. However, a urine output of 6 L/d by postoperative day 6 is high. The observed downtrend in urine output throughout the study, along with apical staining for AQP2 in the collecting duct, suggests that water balance could be maintained without i.v. support with longer follow-up. However, further studies are needed.

In addition to vasopressin-responsive principal cells, the intercalated cells of the collecting duct also appeared functional. The presence of vacuolar-type H+–adenosine triphosphatase B1/2, an adenosine triphosphate–dependent proton pump, on the apical surface of intercalated cells coupled with persistently low urinary pH supports intact urinary acidification in the pig kidney xenograft.

Data from *in vitro* and *in vivo* NHP models support reduced ability of pig renin to cleave human angiotensinogen.[7] Similar findings were observed in the present study. Renin concentrations measured on postoperative days 3 to 7 were the result of pig kidney production. The undetectable plasma renin activity confirmed little ability of the pig renin to cleave human angiotensinogen, although angiotensin II and aldosterone were detected. The ability to maintain blood pressure without use of any inotropes in the absence of native human kidney renin production, combined with measured levels of angiotensin II and aldosterone after porcine kidney xenotransplantation, supports residual RAAS activity. However, most kidney transplants in living persons do not involve bilateral native nephrectomies, and, as such, in the setting of phase 1 clinical trials of porcine kidney xenotransplantation in living persons, RAAS activity will be maintained and hypoaldosteronism and hypotension will be avoided. Renin and aldosterone levels are persevered in patients on hemodialysis for at least 27 months.[19]

The PTH axis principally defends the body's active form of circulating calcium in the blood (e.g., ionized calcium). Because the body's primary stores of calcium are in the form of hydroxyapatite located in bone, PTH has secondary effects on bone mineralization and interacts with vitamin D and phosphate balance. In kidney failure, a combination of vitamin D deficiency and reduced urinary phosphate excretion results in pathologic hyperparathyroidism, which is primarily treated by administering activated vitamin D analogs (e.g., calcitriol). Kidney allotransplantation typically restores PTH levels to normal. In pig-to-NHP xenotransplantation experiments, mild alterations in serum calcium and phosphate levels were observed months after xenotransplantation.[20] In contrast, in our human decedent study of pig-to-human xenotransplantation, PTH levels followed ionized calcium levels appropriately. More important, although serum phosphate levels remained high in the recipient, fractional excretion of phosphorus averaged 29% in the last 3 days of the study, which is above the normal human fractional excretion of phosphate of 5% to 20%. Taken together, these data support preserved PTH signaling with appropriate urinary phosphorus excretion in the days following xenotransplantation.

Our 7-day study aimed at exploring the physiological function of the pig kidney within a human. In the study, nephrotic-range proteinuria was observed. Peak levels of proteinuria were in the first 24 hours at 8.9 g/d and down trended to 3.2 g/d by postoperative day 6. Proteinuria soon after xenotransplantation may indicate early antibody-mediated injury.[21] Although still being explored, little evidence for antibody-mediated rejection was seen. Other possible explanations for proteinuria could be hyperfiltration related to differences in mean arterial blood pressure between the donor pig and recipient human or factors affecting glomerular permeability. Further studies to fully explore the cause of the proteinuria are planned in future decedent studies.

Although our study has many strengths, it does have limitations. Specifically, the study occurred over a relatively short duration, which did not allow the opportunity to assess long-term kidney function. Moreover, the human decedent was brain dead, and as such, it is possible that our results have been confounded by the untoward catabolic state observed in brain death, requiring additional support to replace reduced pituitary function and quell the cytokine storm. Standard brain death management includes low-dose vasopressin and

high-dose corticosteroids, which can affect kidney water handling, particularly in the setting of hyperglycemia with osmotic diuresis.

In summary, we have demonstrated, for the first time, porcine kidney xenograft physiology in a human. More important, these results can be leveraged to develop protocols for phase 1 studies in living persons, with the ultimate goal of establishing pig-to-human kidney xenotransplantation as a feasible option to expand kidney transplantation amidst a human organ shortage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA STATEMENT

Our data are from a single individual and are presented in raw form in the article and its figures. All raw data behind the graphs within the article were provided to the journal.

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Translational Statement

Our work, for the first time in medical history, describes the innate renal physiology of a porcine kidney within the human body, specifically in regard to its ability to regulate mineral and bone metabolism, regulate the renin-angiotensin-aldosterone system, regulate water balance, secrete acid, and process both endogenous and exogenous solutes appropriately. Although preclinical in nature, our in-human study paves the way for future clinical study of pig-to-human kidney xenotransplantation in living persons.

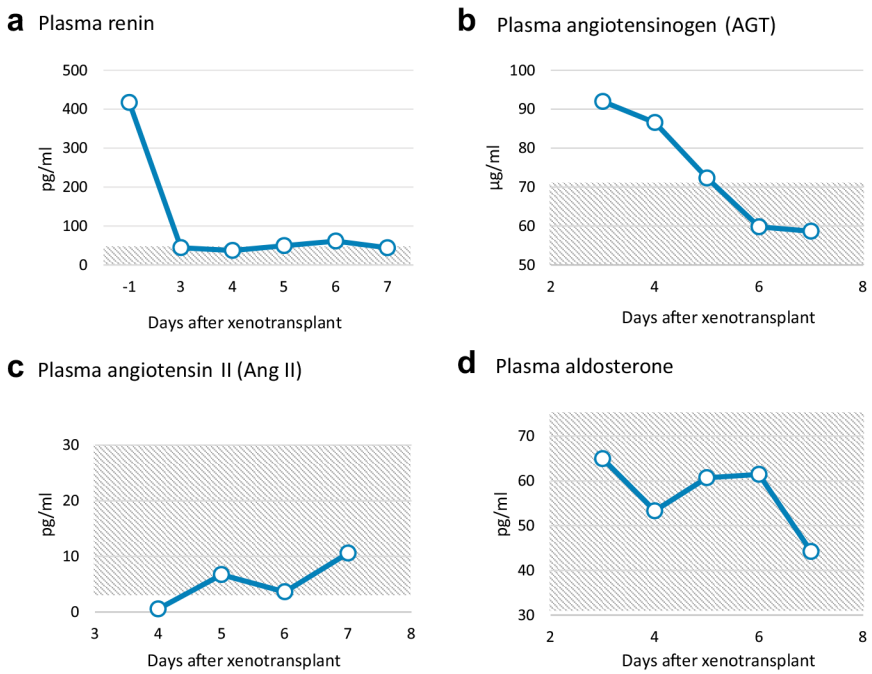

Figure 1 l. Renin-angiotensin-aldosterone system.

Xenotransplant recipient hormone plasma concentrations over time; shaded areas represent normal human ranges for each hormone. (a) Renin (pg/ml): normal, <45.7 pg/ml. Plasma renin activity was <0.6 ng/ml per hour at all time points. (**b**) Angiotensinogen (µg/ml); 71 µg/ml is the upper limit of normal.[12] (**c**) Angiotensin II (pg/ml): normal range, 3 to 30 pg/ml.[13] (**d**) Aldosterone (pg/ml) normal range, 31 to 354 pg/ml.

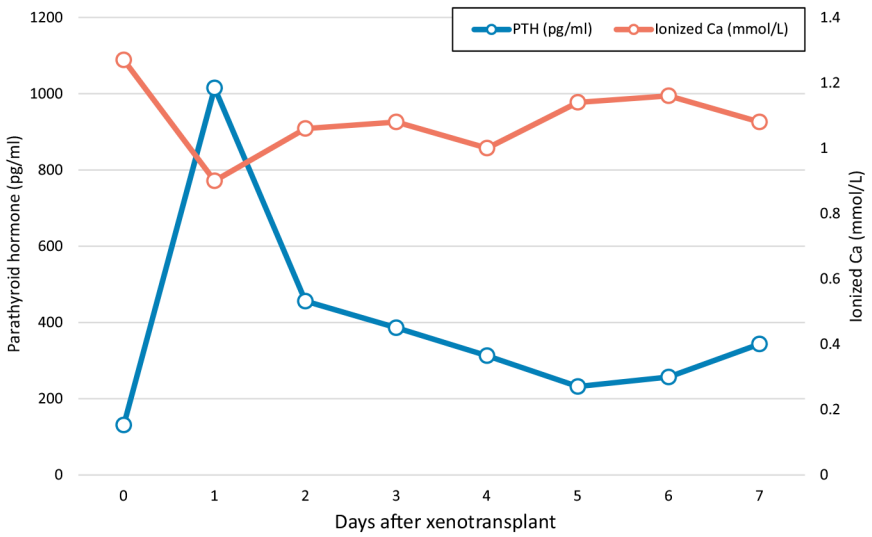

Figure 2 |. Parathyroid hormone (PTH) levels and ionized calcium (Ca) levels in decedent following xenotransplantation.

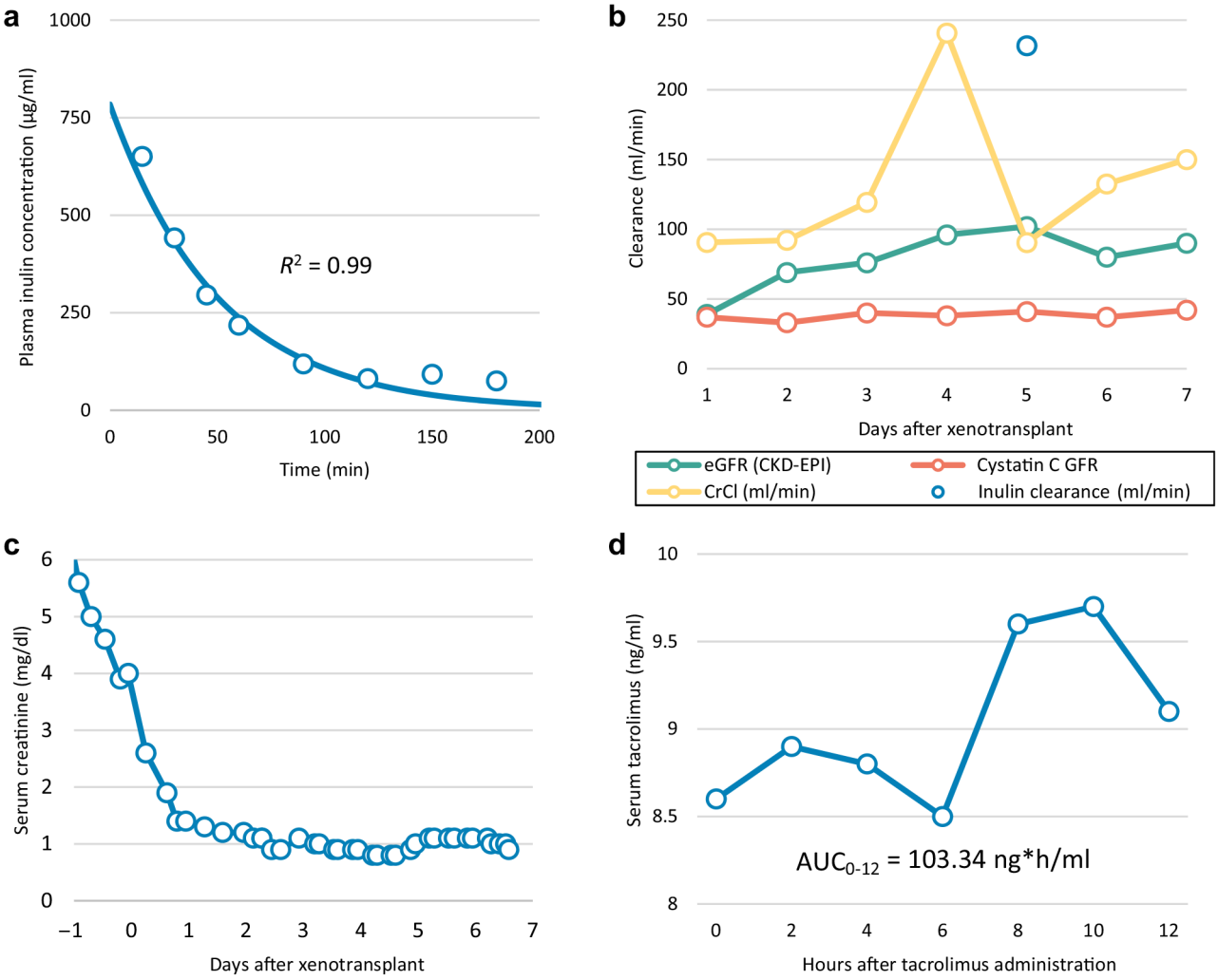

Figure 3 |. Renal clearance physiology.

(**a**) Inulin decay curve, concentration at timed intervals after a 10-g bolus injection (see Supplementary Figure S1 for method). (**b**) Pig kidney clearance grouped by method of measurement. (**c**) Serum creatinine trend. (**d**) Tacrolimus pharmacokinetics. AUC_{0-12}, area under curve 0–12 hours; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CrCl, creatine clearance; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate.

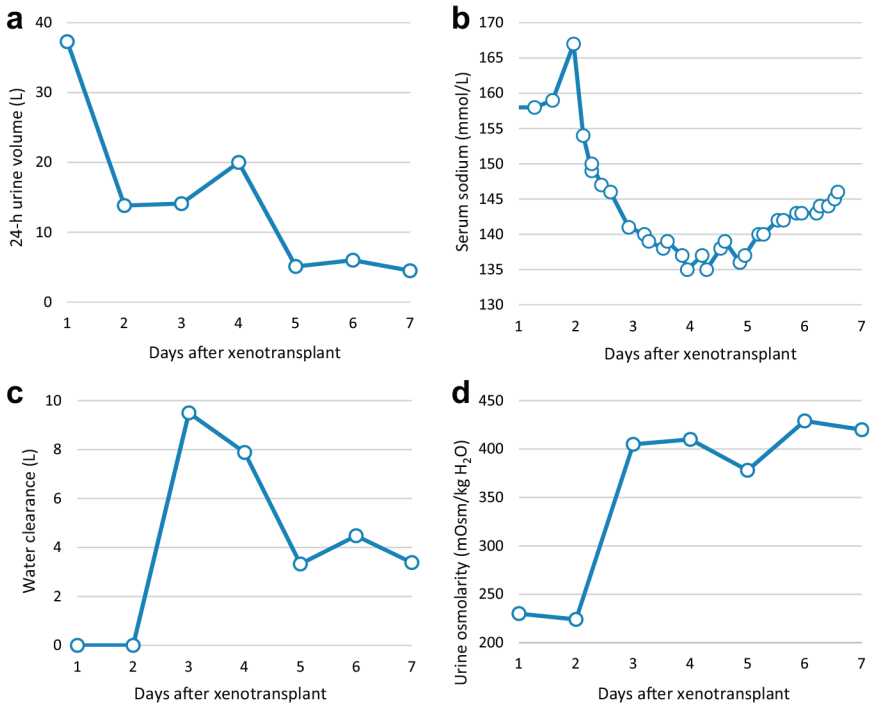

Figure 4 |. Water and sodium balance.

(**a**) Decedent's daily urine output after xenotransplantation (L). Intraoperative furosemide, 100 mg, and mannitol, 25 g, were administered i.v. prior to reperfusion. (**b**) Serum sodium. (**c**) Water clearance after xenotransplantation (L). (**d**) Urine osmolarity (mOsm/kg H_2O).

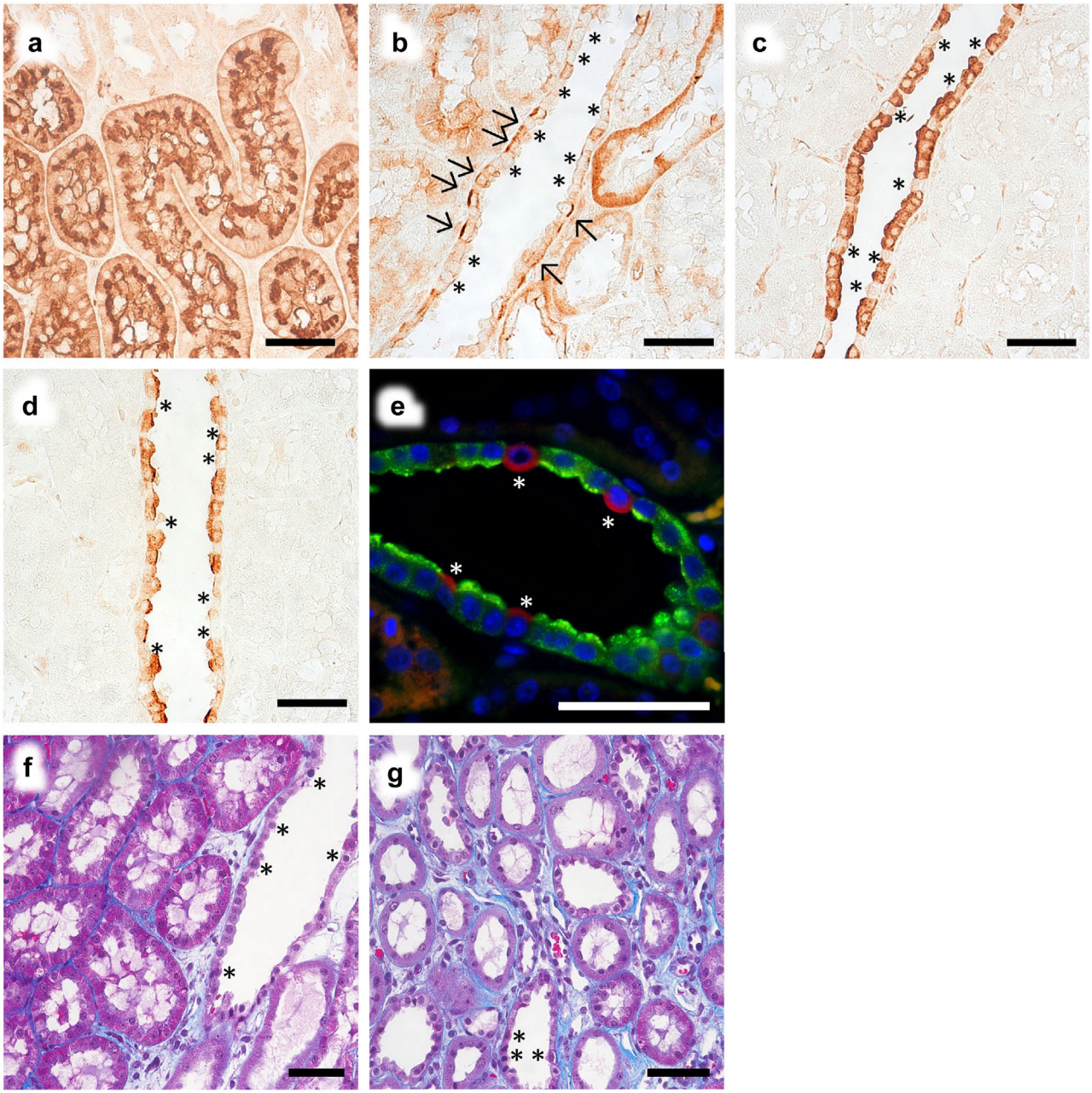

Figure 5 |. Aquaporin (AQP) expression in the 10 gene-edited xenokidney.

(**a**) AQP1 in the apical side of the proximal tubule. (**b**) AQP4 in the basolateral membrane of the principal cells of the collecting duct. Arrows indicate principal cells positive for AQP4. (**c**) AQP2 in the apical membrane of the principal cells. (**d**) AQP2 phosphorylation S256, a known activated form of AQP2, is also expressed in the principal cells. (**e**) Immunofluorescent labeling of principal cells with AQP2–488– (green) and vacuolar-type H+–adenosine triphosphatase B1/2 (V-ATPase)–positive staining of intercalated cells (red); see Supplementary Figure S4 for negative control. (**f**) Cortex and (**g**) medulla: representative trichrome-stained sections with proximal tubules (PTs) and collecting ducts lined by pale

staining principal cells and rare darkly stained intercalated cells. Asterisks (*) denote intercalated cells. Bar = 50 μm.