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Study on the Effects of Water Deprivation Stress on the Ex-pression of Genes Related to the Glomerular Filtration Barrier in Rabbits

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Abstract

The *Lepus yarkandensis* exhibits exceptional urine concentrating ability and unique renal structural features under normal conditions, representing physiological adaptations to arid environments. However, it remains unclear whether such adaptations are genetically fixed traits or inducible phenotypic plasticity. This study aimed to investigate whether domestic rabbits (*Oryctolagus cuniculus*), a closely related species from non-arid habitats, possess latent adaptive potential in their kidneys. We established an acute water deprivation model in healthy adult rabbits and examined mRNA expression changes of key glomerular filtration barrier genes (Nephrin, Podocin, CD2AP) using molecular techniques. Results showed that prolonged water deprivation led to significant weight loss and increased urine osmolality. Nephrin and Podocin mRNA expression was significantly upregulated, and these changes correlated with urine osmolality. These findings reveal the molecular response patterns of the glomerular filtration barrier in rabbits under water restriction, providing comparative data for understanding the drought adaptation mechanisms in *Lepus yarkandensis*.

1. INTRODUCTION

The mammalian kidney plays a pivotal role in maintaining water and electrolyte homeostasis, a function that is particularly critical for species inhabiting arid environments(Mbassa, 1988). *Lepus yarkandensis*, endemic to the arid and semi-arid regions of the Tarim Basin in Xinjiang, China, has evolved remarkable physiological adaptations to chronic water scarcity. Under normal conditions, this species exhibits an exceptional urine concentrating ability and distinct renal structural features, which are considered evolutionary adaptations to its xeric habitat(Zhang et al., 2019). Previous studies have shown that the expression levels of aquaporins (*AQP1*, *AQP2*, *AQP3*, and *AQP7*) and certain transcription factors (*HIF1A*, *NFATc1*, and *NF-kB1*) in the kidneys of *Lepus yarkandensis* are significantly higher than those in domestic rabbits, which facilitates water reabsorption by the kidneys (Luo et al., 2022; Zhang et al., 2019). Simultaneously, the differential expression of cytochrome P450 enzymes (such as *CYP2E1*) in the kidneys of the *Lepus yarkandensis* also suggests a stronger capacity for renal water reabsorption (J. Zhang et al., 2025). These molecular-level findings collectively outline a finely tuned regulatory network for drought adaptation at the renal tubular level in the *Lepus yarkandensis*. A fundamental question in evolutionary physiology is whether such specialized traits represent genetically fixed adaptations or inducible phenotypic plasticity(Lind et al., 2015). Phenotypic plasticity enables organisms to rapidly adjust their physiological functions in response to environmental changes, which is of significant adaptive importance for coping with fluctuating water resources. To investigate this, the domestic rabbit (*Oryctolagus cuniculus*), a closely related species from a non-arid evolutionary background, serves as an ideal comparative model. It is worth noting that European rabbits exhibit flexible phenotypic plasticity in renal physiology, enabling them to adapt to different bioclimatic regions; for example, domestic rabbit populations inhabiting arid islands in South Africa exhibit increased relative medullary thickness and a higher predicted maximum urine concentration capacity (Tate & Marco, 2015). By subjecting domestic rabbits to controlled water deprivation stress, we can assess the potential adaptive capacity of their renal system and determine whether the complex water-saving mechanisms observed in the *Lepus*

yarkandensis can be acutely induced in a species that has not undergone natural selection under conditions of extreme drought.This research strategy not only helps elucidate the evolutionary origins of the *Lepus yarkandensis* specialized traits but also provides an experimental framework for understanding the limits of the renal system's response to environmental stress.

Previous experiments have shown that water deprivation can significantly induce renin expression in rabbit kidneys, thereby confirming the validity of rabbits as a model for studying dehydration stress. Based on this, the present study further focuses on GFB associated podocyte markers to elucidate the molecular plasticity of this structure under water-deprived conditions (do Nascimento et al., 2022).The glomerular filtration barrier (GFB), a highly specialized structure comprising podocytes, the glomerular basement membrane, and endothelial cells, is crucial for regulating the primary filtrate's volume and composition(Miran et al., 2025). Key podocyte specific proteins, such as Nephrin, Podocin, and CD2AP, are integral to the integrity and function of the slit diaphragm, which constitutes the final size-selective barrier. As a member of the stomatin family, Podocin localizes to the insertion sites of the gap junction membrane in podocyte podocytes and interacts with CD2AP and Nephrin via its COOH-terminal domain, collectively forming the structural scaffold of the gap junction membrane (Schwarz et al., 2001).Changes in the expression of these proteins can directly influence the permeability of the GFB, thereby affecting water and solute handling by the kidney—a primary response mechanism to hydration challenges(Coward et al., 2005; Huber et al., 2023; Schwarz et al., 2001). However, current research on the dynamics of glomerular filtration barrier protein expression under water stress conditions remains limited, and the inducible responses in non-drought-adapted species, in particular, remain unclear.

This study aimed to elucidate the molecular response patterns of the GFB in domestic rabbits under acute water deprivation stress. We established a water deprivation model and employed a combination of hematological, biochemical, urinalysis, and molecular techniques—including qRT-PCR and Western blotting—to analyze the expression of critical GFB associated genes and proteins (Nephrin, Podocin, CD2AP, Laminin, WT1). Furthermore, transcriptomic sequencing (RNA-seq) and Gene Set Enrichment Analysis (GSEA) were conducted to explore broader changes in renal pathways. Our findings demonstrate that water deprivation triggers significant physiological changes, including hemoconcentration and maximal activation of urine concentrating capacity. At the molecular level, we observed a distinct upregulation of specific podocyte genes, suggesting a compensatory response aimed at reinforcing the filtration barrier under stress. These results provide comparative insights into the drought adaptation mechanisms of the kidney, highlighting the differential adaptive capacities between a highly specialized species like *Lepus yarkandensis* and a generalist model like the domestic rabbit. This work enhances our understanding of the molecular basis of renal environmental adaptation and the plasticity of the glomerular filtration apparatus in response to water stress.

2. MATERIALS AND METHODS

2.1 Experimental Materials

Sixteen healthy adult male rabbits (*Oryctolagus cuniculus*), weighing 2.0-2.5 kg, were provided by the Experimental Station of the College of Animal Science and Technology, Tarim University. The animals were housed in spacious and well-ventilated individual cages under a 12h light/12h dark cycle. The housing conditions complied with the national standard for “Laboratory Animal-Environment and Housing Facilities”. Throughout the experiment, the animals’ survival status and physiological conditions were regularly monitored, including feed intake, activity, and signs of disease. All animal capture and experimental procedures were approved by the Animal Ethics and Use Committee of Tarim University (Approval No. 2022015), and all methods were carried out in accordance with relevant local laws, guidelines, and policies on animal welfare.

2.2 Experimental Grouping and Water Deprivation Stress Treatment

2.3 Blood Biochemical Analysis

To minimize animal stress, rabbits were gently restrained with a soft towel to ensure comfort and reduce struggling. Whole blood (n = 8) was collected from the marginal ear vein and immediately transferred into lithium-heparin vacuum blood collection tubes. Key biochemical parameters, including blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), total bilirubin (TBIL), globulin (GLOB), urea (U-REA), albumin-to-globulin ratio (A/G), and BUN-to-creatinine ratio (B/C), were measured using a fully automated multifunctional biochemical analyzer.

2.4 Hematological Analysis

Whole blood was promptly transferred into EDTA-K_2 vacuum tubes for hematological analysis. Parameters including total white blood cell count (WBC), total red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and mean corpuscular hemoglobin (MCH) were determined using a fully automated five part differential hematology analyzer.

2.5 Urinalysis

Urine was collected using sterile trays placed under the rabbit cages. Urine total protein (UTP), urine specific gravity (USG), and urine pH (UpH) were measured using URIT urine test strips, and analysis was performed with a veterinary urine analyzer.

2.6 Osmotic Pressure Analysis

Whole blood containing EDTA-K_2 was centrifuged at 3500 rpm for 12 minutes, re-sulting in three layers: (1) bottom layer (red blood cells), (2) middle layer (white blood cells and platelets, referred to as the buffy coat), and (3) upper layer (plasma, a pale-yellow transparent fluid). The supernatant (plasma) was collected. Urine was collected in plain tubes without additives. Osmolality of plasma and urine was measured using a freezing point osmometer.

2.7 RNA Extraction and cDNA Synthesis

Total RNA was extracted from kidney tissues of domestic rabbits using the Trizol method. Briefly, 80–100 mg of kidney tissue was ground in liquid nitrogen, lysed with 1 mL of Trizol reagent, and incubated at room temperature for 5 minutes. Chloroform was added for phase separation, and the aqueous phase was collected after centrifugation. RNA was precipitated with isopropanol, washed with 75% ethanol, dissolved in 30 μL of DEPC-treated water, and stored at -80°C. cDNA was synthesized using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit.

2.8 Real-Time Quantitative PCR (qRT-PCR)

Primers were designed using Primer 5, with GAPDH serving as the reference gene.The primer sequences are shown in Table 1. Differential gene expression was validated by qRT-PCR using the $2^{-\Delta\Delta Ct}$ method.

Table 1
Target Gene Sequences and Internal Control Primers

Gene	Primer Sequences(5’-3’)
Nephrin	Forward: GGGGTTCTGTGGACCTAGTC
	Reverse: CGTGGACCCCTTTGAGATCT
Podocin	Forward: GCCAGAGGAAGGTACCAAGT
	Reverse: GGAAGCAGATGTCCCAGTCT
CD2AP	Forward: CCTGAACAACAAGTCGGGAC
	Reverse: CGGAGAGGGTAAAGGTGAGG
Laminin	Forward: GACAAGGGCTGCACACAC
	Reverse: CCCAAACTGATAGGCGTCAC
WT1	Forward: GAATACACACCCATGGCGTC
	Reverse: CGGGTAAGCACACATGAAGG
GAPDH	Forward: GGTTGTCTCCTGCGACTTCA
	Reverse: TGGTCCAGGTTTCTTACTCC

2.9 Quantitative Western Blotting

Protein extraction was performed using RIPA lysis buffer containing 1 mM PMSF and a protease inhibitor mixture. Kidney tissues were lysed at 4°C for 30 minutes with vortexing every 10 minutes, followed by centrifugation at 12,000 g for 15 minutes at 4°C. The supernatant was collected as total protein. Protein concentration was measured using the BCA method. A total of 30 μg protein per lane was mixed with 5× SDS-PAGE loading buffer, denatured at 95°C for 5 minutes, and separated on 10% or 12% SDS-PAGE gels (80 V for 30 minutes, then 120 V until the bromophenol blue reached the bottom of the gel).

Proteins were transferred to PVDF membranes using the wet transfer method (300 mA, 90 minutes). Membranes were blocked with 5% skim milk in TBST for 1 hour at room temperature, incubated with primary antibodies (Nephrin 1:1000, Podocin 1:800, CD2AP 1:200, Laminin 1:200, WT1 1:200, diluted in TBST containing 5% BSA) overnight at 4°C. After washing three times with TBST (10 minutes each), membranes were incubated with HRP-conjugated secondary antibodies (1:5000 in TBST) for 1 hour at room temperature. Membranes were washed again three times with TBST, developed with ECL chemiluminescent reagent, and detected using a Tanon-5200 chemiluminescence imaging system. Band intensity was analyzed using ImageJ software, with GAPDH as the internal reference.

The sources of primary antibodies were as follows: Nephrin antibody (Cat# 66970-1-Ig, Proteintech); Podocin antibody (Cat# 5863808, UnivBio); CD2AP antibody (Cat# DF2298, Affinity Biosciences); Laminin 2α antibody (Cat# DF13275, Affinity Bio-sciences); WT1 antibody (Cat# 12609-1-AP, Proteintech).

2.10 Statistical Data Analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0). To assess the statistical significance of differences among experimental groups, one-way analysis of variance (ANOVA) was used. Prior to ANOVA, data normality was evaluated using the Shapiro–Wilk or Kolmogorov–Smirnov test, and homogeneity of vari-ances was verified using Bartlett's or Brown–Forsythe test. If the assumptions were met, one-way ANOVA was performed. When ANOVA indicated a significant overall effect ($p < 0.05$), post-hocmultiple comparison tests (Tukey's HSD test) were used for pairwise comparisons between groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3. RESULTS AND DISCUSSIONS

3.1 Comparative Analysis of Hematological Parameters between Control and Water-Deprived Rabbits

Hematological parameters serve as critical indicators of physiological status and environmental adaptation (Maceda-Veiga et al., 2015). Red blood cell count (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) are primary metrics for oxygen-carrying capacity, typically increasing under conditions of hypoxia or elevated metabolic demand (Mairbäurl, 2013). White blood cell count (WBC) and its differentials (e.g., neutrophils, lymphocytes) are important markers of immune system activity and inflammatory status. Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), reflect red blood cell volume and hemoglobin content, providing insights into nutritional status, hydration, or potential pathological changes.

To investigate the physiological differences between control and water-deprived rabbits, a systematic analysis of their blood biochemical parameters was conducted. As shown in Table 2, no significant

differences ($P > 0.05$) were observed between the two groups for multiple indicators, including serum calcium (Ca), uric acid (UA), blood glucose, and total bilirubin.

However, no significant differences ($P > 0.05$) were observed between the two groups for multiple parameters, including uric acid (UA), total bilirubin (TB), and serum calcium (Ca). Further analysis revealed that total cholesterol (TC), triglycerides (TG), and creatinine (Cr) levels also showed no statistically significant intergroup differences ($P > 0.01$). It is noteworthy that the expression levels of creatine kinase (CK) and alanine aminotransferase (ALT) similarly exhibited no significant differences between the two groups ($P > 0.001$).

Hematological analysis results (Table 3) showed that parameters such as eosinophil count (Eos#), monocyte percentage (Mon%), and red cell distribution width-coefficient of variation (RDW-CV) showed no significant differences between the two groups ($P > 0.05$). However, the water-deprived rabbits exhibited significantly higher red blood cell count (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) compared to the control group, with differences reaching a highly significant level ($P < 0.001$). Concurrently, the platelet count (PLT) in the water-deprived group was significantly lower than that in the control group ($P < 0.01$).

The synchronous and significant increases in BUN, Cr, and total protein in water-deprived rabbits provide the most direct biochemical indication of hemoconcentration. Due to the cessation of water intake, rabbits could only conserve water by reducing urine excretion; however, their limited renal concentrating capacity led to a decrease in plasma volume and relative concentration of non-water-soluble components in the blood, such as proteins and nitrogenous waste products. This pattern closely resembles the elevated BUN and Cr levels observed in *Lepus yarkandensis* under normal conditions, suggesting that the "physiological azotemia" in *Lepus yarkandensis* likely represents a homeostatic state adapted to chronic mild dehydration through long-term evolution, rather than a pathological condition. The marked changes in the erythrocyte system (increased RBC, HGB, HCT) are also a direct result of hemoconcentration and may be further driven by erythropoietin (EPO) release in response to mild hypoxic stress (due to increased blood viscosity). This again aligns with the characteristics of *Lepus yarkandensis*, indicating that enhancing blood oxygen-carrying efficiency is a conserved adaptive strategy in mammals under water restriction (Liao et al., 2026).

Furthermore, changes in metabolic parameters in water-deprived rabbits revealed a stress-induced metabolic remodeling. The significantly elevated total cholesterol (TC) and triglycerides (TG) may reflect a dual adaptation: on one hand, enhanced mobilization and utilization of lipids as a more "water-efficient" energy substrate (yielding more metabolic water per unit of oxygen during oxidation) to reduce dependence on glucose metabolism; on the other hand, possibly due to enhanced lipolysis resulting from reduced food intake or stress. The sharp rise in creatine kinase (CK) strongly suggests muscle catabolism or injury. Under the combined pressure of limited water and energy intake, the body may break down skeletal muscle protein to provide precursors for gluconeogenesis and energy, leading to the release of intramuscular contents, including CK, into the bloodstream. This contrasts with the

substantial decrease in alanine aminotransferase (ALT) activity, which may reflect the liver's prioritization of core metabolic functions over specific transaminase activities under systemic stress. The significant decrease in the albumin-to-globulin ratio (A/G) was primarily due to a greater in-crease in globulin (GLB, mainly immunoglobulins) compared to albumin, possibly indicating stress-induced activation of the immune system or associated with potential mild tissue damage/inflammatory reactions.

Comparing the data from water-deprived rabbits with the baseline data of *Lepus yarkandensis* provides profound insights into the evolutionary mechanisms of drought tolerance. Both show similarities in hemoconcentration (elevated BUN, Cr, TP, RBC, HGB) and high creatine kinase (CK) levels, but the underlying physiological logic may differ. For domestic rabbits, this represents an acute stress response accompanied by marked metabolic disturbances (e.g., dyslipidemia) and potential signs of muscle catabolism. In contrast, for *Lepus yarkandensis*, this constitutes an optimized homeostatic adaptation, likely accompanied by a more stable metabolic profile (e.g., lipid levels) and a muscular system adapted to this physiological state of high CK activity, rather than indicating injury.

Table 2
Blood biochemical parameters in control and water-deprived rabbits

Indicator	Reference Range	Control Rabbits (n = 8)	Water-Deprived Rabbits (n = 8)	t-value	p-value
Serum calcium (Ca) (mmol/L)	2.2–2.7	3.3 ± 0.22	3.63 ± 0.66	1.393	0.194
Uric acid (UA) (mg/dL)	3.6–8.0	11.07 ± 2.08	10 ± 0	1.452	0.182
Blood glucose (G) (mmol/L)	3.9–6.1	14.47 ± 3.71	11.52 ± 5.7	1.334	0.213
Total cholesterol (TC) (mmol/L)	< 5.17	1.31 ± 0.47	42.2 ± 60.47**	2.145	0.049
Triglyceride (TG) (mmol/L)	< 1.7	1.01 ± 0.56	28.59 ± 38.18**	2.281	0.038
Total bilirubin (TB) (μmol/L)	0–20.5	1.17 ± 0.93	7.47 ± 12.76	1.532	0.153
Alkaline phosphatase (ALP) (U/L)	40–150	131.2 ± 12.69	62.33 ± 9.29***	12.394	0.001
Gamma-glutamyl transferase (GGT) (U/L)	0–45	19.63 ± 2.41	2 ± 0***	20.705	0.001
Total protein (TP) (g/L)	60–80	51.99 ± 6.94	84.87 ± 2.03***	12.842	0.001
Albumin (ALB) (g/L)	35–50	35.37 ± 4.7	48.53 ± 2.84***	6.786	0.001
Globulin (GLB) (g/L)	20–35	15.66 ± 3.29	36.4 ± 2.31***	15.221	0.001
Albumin/Globulin ratio (A/G)	1.5–2.5	2.4 ± 0.41	1.34 ± 0.16***	6.423	0.001
Lactate dehydrogenase (LDH) (U/L)	120–250	149.2 ± 47.91	424 ± 140.01***	3.422	0.004
Creatine kinase (CK) (U/L)	24–195	928.67 ± 468.54	3376.67 ± 1079.65***	6.335	0.001
Alanine aminotransferase (ALT) (U/L)	9–50	38 ± 4.9	2 ± 0***	21.91	0.001
AST/ALT ratio	0.8–2.1	1.22 ± 0.86	1.99 ± 0.48**	2.316	0.036
Urea nitrogen (BUN) (mmol/L)	1.8–7.1	7.14 ± 0.34	29.35 ± 13.58***	5.484	0.001
Creatinine (Cr) (μmol/L)	44–115	71.7 ± 12.06	182.55 ± 9.44**	2.566	0.023
BUN/Cr ratio	10–20	161.61 ± 32.12	93.59 ± 42.07***	3.842	0.002

Table 3
Hematological parameters in control and water-deprived rabbits

Indicator	Reference Range	Control Rabbits	Water-Deprived Rabbits	t-value	p-value
White blood cell count (WBC) (×10⁹/L)	3.00–13.50	7.34 ± 2.86	7.48 ± 1.16	0.082	0.945
Neutrophil count (Neu#) (×10⁹/L)	0.50–6.60	2.71 ± 1.68	4.35 ± 2.69	1.065	0.373
Lymphocyte count (Lym#) (×10⁹/L)	1.00–6.80	4.05 ± 1.04	2.6 ± 1.94	1.393	0.266
Monocyte count (Mon#) (×10⁹/L)	0.08–1.51	0.4 ± 0.39	0.36 ± 0.12	0.216	0.853
Eosinophil count (Eos#) (×10⁹/L)	0.00–0.51	0.18 ± 0.09	0.16 ± 0.13	0.284	0.802
Basophil count (Bas#) (×10⁹/L)	0.00–0.76	0 ± 0	0 ± 0	—	—
Neutrophil percentage (Neu%) (%)	14.0–62.0	33.83 ± 14.2	56.4 ± 27.56	2.541	0.0847
Lymphocyte percentage (Lym%) (%)	25.0–82.0	58.9 ± 16.79	36.37 ± 27.05	2.544	0.0843
Monocyte percentage (Mon%) (%)	2.0–15.0	4.53 ± 3.75	5.03 ± 1.94	0.262	0.812
Eosinophil percentage (Eos%) (%)	0.0–6.0	2.63 ± 1	2.1 ± 1.4	0.575	0.614
Basophil percentage (Bas%) (%)	0.0–8.0	0.1 ± 0	0.1 ± 0	—	—
Red blood cell count (RBC) (×10¹²/L)	3.40–6.50	5.66 ± 0.12	8.16 ± 0.27***	16.622	0.001
Hemoglobin concentration (HGB) (g/L)	80–140	121 ± 5.57	168.67 ± 5.51***	11.545	0.001
Hematocrit (HCT) (%)	25.0–42.0	37.2 ± 2.17	52.4 ± 5.6***	5.471	0.005
Mean corpuscular volume (MCV) (fL)	60.0–80.0	65.7 ± 2.63	64.1 ± 5.35	0.523	0.643
Mean corpuscular hemoglobin (MCH) (pg)	19.0–25.0	21.37 ± 0.5	20.7 ± 0.66	1.685	0.194
Mean corpuscular hemoglobin concentration (MCHC) (g/L)	300–360	332.33 ± 19.73	324 ± 23.81	0.572	0.612

Indicator	Reference Range	Control Rabbits	Water-Deprived Rabbits	t-value	p-value
Red cell distribution width-CV (RDW-CV) (%)	10.5–22.0	13.77 ± 0.93	13.43 ± 0.85	0.544	0.635
Red cell distribution width-SD (RDW-SD) (fL)	28.0–65.0	36.63 ± 1.56	34.9 ± 2.82	1.062	0.376
Platelet count (PLT) (×10⁹/L)	100–1250	443.5 ± 43.01	320.5 ± 67.18**	2.453	0.012
Mean platelet volume (MPV) (fL)	4.0–7.8	5.43 ± 0.59	6.03 ± 0.72	1.396	0.262
Platelet distribution width (PDW) (fL)	0.1–30.0	5.77 ± 1.44	9.23 ± 3.95	1.763	0.224
Plateletcrit (PCT) (%)	0.000–0.800	0.17 ± 0.1	0.15 ± 0.09	0.317	0.775

3.2 Comparative Analysis of Body Weight Changes in Control and Water-Deprived Rabbits

During the experiment, the body weight of the control rabbits remained stable with no significant changes. In contrast, the water-deprived group exhibited a progressive decrease in body weight over the course of water deprivation. When the body weight loss exceeded 10%, the rabbits displayed stress-related symptoms such as lethargy and reduced activity. This pattern of weight loss is consistent with the observations reported by da Cruz-Filho in a study of male C57BL/6 mice subjected to 36 hours of water deprivation, indicating that the domestic rabbits had developed a marked response to water deprivation (da Cruz-Filho et al., 2025).

3.3 Comparative Analysis of Urine Osmolality Changes in Control and Water-Deprived Rabbits

Renal urine concentrating ability is a core defense mechanism against dehydration (Agaba et al., 2012). This study confirmed that water deprivation successfully induced a potent urine concentrating response in rabbits. As shown in Table 4, urine osmolality (UOsm) in the water-deprived group significantly increased to 1862.3 ± 124.7 mOsm/kg·H_2O, compared to 715.9 ± 198.5 mOsm/kg·H_2O in the control group. The difference was highly statistically significant ($t = 15.67$, $P < 0.001$). Concurrently, plasma osmolality in the water-deprived group (352.1 ± 18.9 mmol/L) showed a slight, non-significant increase compared to the control group (339.7 ± 23.4 mmol/L, $P = 0.083$). This indicates that under water deprivation, the body prioritizes urine concentration over substantially elevating plasma osmolality to maintain homeostasis.

Consistent with the highly concentrated urine, characteristic changes in urine physicochemical properties were observed in the water-deprived group (Table 5). Specifically, urine specific gravity (USG),

a key indicator of urine solute concentration, was significantly elevated in the water-deprived group (1.028 ± 0.003 vs. 1.012 ± 0.004 in controls, P < 0.001). Due to the high urine concentration, urinary total protein (UTP) concentration also increased significantly (0.22 ± 0.08 g/L vs. 0.05 ± 0.02 g/L, P < 0.001). Furthermore, urine pH was significantly lower in the water-deprived group (6.5 ± 0.4 vs. 8.2 ± 0.5, P < 0.001), suggesting potential compensatory metabolic adjustments. For the qualitative indicator, urobilinogen (URO) was positive (2^{+}) in the water-deprived group but negative in controls, reflecting adaptive changes in liver metabolism and renal excretion under water deprivation.

The urine osmolality of water-deprived rabbits increased sharply from ~ 716 mOsm/kg to ~ 1862 mOsm/kg, representing a rise of more than 2.6 fold. This demonstrates the maximal activation of the renal medullary countercurrent multiplication system driven by antidiuretic hormone (ADH), which constitutes the most critical physiological response for reducing water loss and sustaining life under acute water deficit. Notably, although the achieved concentration limit (1862 mOsm/kg) was significant, it remained substantially lower than the urine osmolality routinely maintained by *Lepus yarkandensis* under normal conditions (~ 2159 mOsm/kg).

The significant increase in urine total protein (UTP) observed in water-deprived rabbits is similar to the pattern seen in *Lepus yarkandensis*. When urine is concentrated to an extremely high level, the protein concentration in the tubular fluid becomes physically elevated. Concurrently, the extremely high medullary osmotic gradient may impose physical stretch or osmotic stress on renal tubular epithelial cells, potentially leading to a slight increase in paracellular permeability or saturating the reabsorptive capacity for the small amount of protein normally filtered through the glomerulus, thereby resulting in overflow proteinuria. This likely does not indicate structural damage to the glomerular filtration barrier, but rather represents a functional phenomenon accompanying the extreme physiological state.

In this study, a drought-stress model in rabbits was established using a water deprivation protocol, with the rate of body weight loss and urine osmolality serving as model evaluation indicators. The results showed that prolonged water deprivation led to progressive weight loss and a significant increase in urine osmolality. The moderate-stress group (4-day water deprivation) exhibited a 10.3% reduction in body weight and a urine osmolality exceeding 1200 mOsm/kg·H_2O, which aligns with the physiological characteristics of a drought-stress model.

Table 4
Urine and plasma osmolality in control and water-deprived rabbits.

Parameter	Reference Range	Control Rabbits	Water-Deprived Rabbits	t-value	p-value
Plasma osmolality (mmol/L)	340–360	339.7 ± 23.4	352.1 ± 18.9	1.84	0.083
Urine osmolality (mmol/L)	50–1400	715.9 ± 198.5	1862.3 ± 124.7	15.67	< 0.001

Table 5
Urinalysis results in control and water-deprived rabbits.

Parameter	Reference Range	Control Rabbits	Water-Deprived Rabbits	t-value	p-value
Urine total protein (g/L)	0–0.15	0.05 ± 0.2	0.22 ± 0.08	6.12	< 0.001
Urine specific gravity (USG) (g/cm³)	1.003–1.035	1.012 ± 0.004	1.028 ± 0.003	9.87	< 0.001
Urine pH	0–14	8.2 ± 0.5	6.5 ± 0.4	8.45	< 0.001
Urobilinogen	“–” – “4+”	“–”	“2+”	-	-

3.4 Expression levels of glomerular filtration barrier proteins in response to water deprivation

To investigate the effects of water deprivation on podocyte function, we examined the expression levels of key structural and functional genes and proteins in podocytes. As shown in Fig. 1(A-E), the mRNA expression of podocyte specific protein genes (Nephrin, Podocin, CD2AP, Laminin, WT1) was significantly altered following water deprivation treatment. Among them, the transcriptional levels of Nephrin, Podocin, and WT1 were significantly downregulated (*p < 0.05), while the expression of CD2AP and Laminin showed a decreasing trend without reaching statistical significance (ns).

At the protein level, Western blot results further corroborated the gene expression trends (Fig. 1(F-O)). Compared with the control group, the protein expression of Nephrin, Podocin, and WT1 was significantly reduced in the water-deprived group (*p < 0.05), consistent with the changes at the mRNA level. The protein levels of CD2AP and Laminin also displayed a downward tendency, but without statistical significance. These findings suggest that water deprivation may specifically affect the expression of key podocyte functional proteins through transcriptional and post-transcriptional reg-ulatory mechanisms, thereby potentially compromising podocyte structure and the integrity of the glomerular filtration barrier.

Under short-term water deprivation stress, the rabbit body enhances the concentrating function of the kidneys to reduce water loss. During this period, the upregulation of Nephrin and Podocin gene expression may strengthen the stability of the slit diaphragm complex, decrease the permeability of the filtration barrier, reduce water filtration in the primary urine, and thereby improve renal water reabsorption efficiency (Schwarz et al., 2001). In contrast, under long-term severe stress, severe water deficiency may lead to renal tissue damage, resulting in downregulation of Nephrin and Podocin gene expression. This phenomenon could be a self-protective mechanism to prevent filtration barrier dysfunction caused by excessive stress (Woodard et al., 2004).

The expression of the CD2AP gene showed a continuous upward trend, which differed from the regulatory patterns of Nephrin and Podocin. As an adaptor protein, CD2AP not only participates in

maintaining the stability of the Nephrin-Podocin com-plex but also plays a role in cell signaling and apoptosis regulation. The sustained up regulation of CD2AP expression may contribute to drought adaptation through two pathways: first, by enhancing the anchoring of Nephrin and Podocin proteins to offset the negative effects of their decreased expression under severe stress, thereby preserving the basic function of the filtration barrier; second, by regulating apoptotic signals to protect podocytes from damage induced by drought stress and ensure the continuity of renal filtration function (Li et al., 2000; Shih et al., 1999). These findings suggest that CD2AP may play a more critical protective role in drought adaptation.

Both Laminin and WT1 genes exhibited downregulation trends under water deprivation stress. The downregulation of Laminin may affect the structural integrity of the glomerular basement membrane, while the downregulation of WT1 may inhibit normal differentiation and functional maintenance of podocytes. However, the coordinated upregulation of Nephrin, Podocin, and CD2AP may partially counteract these negative effects, helping to maintain the relative stability of the filtration barrier (Khegay, 2023; Pritchard-Jones et al., 1990).

During drought adaptation in rabbits, the kidneys employ a dual mechanism to conserve water: first, by upregulating aquaporin genes to enhance water reabsorption in renal tubules; second, by modulating filtration barrier-related genes to reduce glomerular filtration efficiency. These two mechanisms work synergistically to maintain the body's water balance (Haraldsson et al., 2008; Pavenstädt et al., 2003). The regulatory patterns of filtration barrier genes revealed in this study complement the molecular network of animal drought adaptation and provide a new perspective for a comprehensive understanding of the environmental adaptive functions of the kidneys.

3.5 Transcriptomic GO Enrichment Analysis Reveals Stress Response, Apoptosis, and Structural Remodeling in the Kidney under Water Deprivation Stress

RNA sequencing (RNA-seq) analysis revealed 48 differentially expressed genes (17 upregulated and 31 downregulated, criteria: qValue < 0.05 and $|\log_2 \text{FoldChange}| > 1.0$) in kidney tissues of water-deprived rabbits compared to the control group (Fig. 2). Among these, key filtration barrier genes—CD2AP, Podocin, and Nephrin—were upregulated, while Laminin and WT1 were downregulated. GO enrichment analysis showed significant upregulation of terms such as "positive regulation of apoptotic process" and "positive regulation of programmed cell death," suggesting that water deprivation stress may induce stress-related injury or apoptosis in glomerular epithelial cells (podocytes).

Simultaneously, upregulated terms such as "response to corticotropin-releasing hormone" and "cellular response to corticotropin-releasing hormone stimulus" indicate that kidney tissue, under systemic dehydration stress, is regulated by the hypothalamic–pituitary–adrenal (HPA) axis, activating glucocorticoid-related signaling pathways to maintain water–electrolyte balance and metabolic homeostasis.

At the cellular component level, significantly enriched terms included "extracellular matrix" and "laminin complex." These results imply that water deprivation stress may trigger structural remodeling of the glomerular basement membrane and podocyte slit diaphragm, thereby modulating the function of the renal filtration barrier. This aligns with earlier Western blot results showing changes in Nephrin and Podocin expression: upregulation of these slit diaphragm proteins during short-term water deprivation may enhance the mechanical stability and impermeability of the barrier, whereas their downregulation under prolonged severe water deprivation may reflect the onset of structural damage in renal tissue.

Molecular function enrichment results revealed significant upregulation of terms such as "signaling receptor binding," "cell adhesion molecule binding," and "nuclear glucocorticoid receptor binding," further indicating that water deprivation stress activates receptor-mediated signaling regulation and glucocorticoid dependent stress response mechanisms. These changes in molecular functions collectively contribute to maintaining the physiological activity of glomerular cells and their ability to withstand osmotic stress.

3.6 GSEA Pathway Analysis Reveals Water Deprivation-Induced Immune-Inflammatory Activation and Enhanced Energy Metabolism

GSEA enrichment analysis demonstrated significant enrichment of multiple energy metabolism related pathways in the water-deprivation group, including “Citrate cycle (TCA cycle)” and “Oxidative phosphorylation.”(Fig. 3) This indicates a substantial enhancement of energy metabolism in renal tissue under water-deprivation conditions, likely as an adaptive response to osmotic pressure changes and metabolic stress. The involvement of pathways such as “Peroxisome” and “Thermogenesis” further suggests increased reactive oxygen species (ROS) metabolism, consistent with elevated oxidative stress levels during water deprivation.

Moreover, multiple immune- and inflammation-related pathways were significantly enriched, including “Cytokine–cytokine receptor interaction,” “NF-kB signaling pathway,” “TNF signaling pathway,” and “T cell receptor signaling pathway.” The activation of these pathways indicates that water deprivation stress not only triggers cellular stress signaling in the kidney but may also induce excessive immune activation, thereby promoting local inflammatory responses and cellular injury processes in glomeruli. Such immune-mediated responses could further exacerbate dysfunction of the filtration barrier, aligning with the observed downward trend in Nephrin and Podocin expression in Western blot results.

Notably, the enrichment of the “Cell adhesion molecules (CAMs)” pathway is consistent with the GO term “cell adhesion molecule binding,” suggesting dynamic regulation of podocyte adhesion structures under water deprivation. The sustained upregulation of the CD2AP gene may reflect its compensatory role in podocyte cytoskeletal remodeling and adhesion stabilization under stress. Specifically, CD2AP may help maintain the integrity of the cytoskeleton membrane linkage complex, thereby mitigating the structural imbalance caused by the decline of Nephrin and Podocin.

3.7 Activation of the Pentose Phosphate Pathway Serves as the Core Metabolic Adaptation for Alleviating Oxidative Stress

Multiple upregulated enzymes annotated in the figure (marked by orange-red boxes) include 3.1.1.17 (6-phosphogluconolactonase) and 3.1.1.31 (6-phosphogluconate dehydrogenase). Both are located in the oxidative phase of the pentose phosphate pathway (PPP) and are involved in the oxidation of glucose-6-phosphate to ribu-lose-5-phosphate (Ribulose-5P) with concomitant generation of NADPH. This is consistent with the dehydration adaptation observed in the *Xenopus laevis*, in which kidney cells increase their capacity to produce NADPH under water deprivation stress to counteract oxidative stress (B. Zhang et al., 2025).

Enzymes such as 5.3.1.6 (Ribose-5-phosphate isomerase) and 2.2.1.2 (Transketolase, TKT) participate in the non-oxidative phase, catalyzing the interconversion between pentose phosphates and glycolysis intermediates (fructose-6-phosphate and glycer-aldehyde-3-phosphate). This suggests dynamic crosstalk and adaptive remodeling between energy metabolism pathways under water deprivation conditions.

Furthermore, 5.4.2.7 / 5.4.2.2 (Phosphopentomutase) catalyzes the interconversion of ribose-5-phosphate and ribose-1-phosphate, linking PPP with purine and pyrimidine metabolism. Its upregulation implies activation of nucleotide metabolism, which may support DNA repair or intracellular signaling under stress.

The upregulation of these enzymes across key nodes of PPP indicates a systemic activation of the pathway, particularly in NADPH production and ribose metabolism. NADPH serves as a crucial electron donor for the glutathione reductase system, thereby mitigating the accumulation of reactive oxygen species (ROS) induced by water-deprivation. Additionally, PPP supplies reducing equivalents for lipid synthesis and membrane repair, which is essential for renal cells coping with osmotic changes. Meanwhile, ribose-5P provides precursors for nucleotide synthesis, supporting cellular repair and renewal in renal tissue.

Combined with the significant enrichment of the "oxidative phosphorylation" and "peroxisome" pathways in the previous GSEA results, it can be inferred that renal tissue under water deprivation stress activates both PPP and mitochondrial energy metabolism pathways cooperatively to alleviate oxidative stress and maintain cellular homeostasis.

4. CONCLUSION

By subjecting domestic rabbits to water deprivation stress, we reversely validated the inducibility and functional significance of certain adaptive traits described above. Acute water shortage triggered emergency mechanisms in domestic rabbits: their blood exhibited hemoconcentration (elevated RBC, HGB, HCT, BUN, and Cr levels), maximal activation of urine concentrating capacity (significantly increased urine osmolality, though still lower than the baseline level of *Lepus yarkandensis*), and signs of

metabolic stress (elevated blood lipids and a sharp increase in CK). More importantly, the expression of key genes in the renal filtration barrier (Nephrin, Podocin, CD2AP) was significantly upregulated, partially resembling the constitutive expression pattern observed in *Lepus yarkandensiss*. However, the expression trends of Laminin and WT1 were not entirely consistent with those in *Lepus yarkandensiss*. Transcriptomic analysis further demonstrated that water deprivation stress activated stress response pathways, apoptotic signaling, immune-inflammatory pathways, and metabolic reprogramming processes such as the pentose phosphate pathway in the kidneys of domestic rabbits. These results indicate that, when facing acute water crisis, domestic rabbits can attempt to enhance renal water retention by inducing the expression of certain filtration barrier-related genes. Nevertheless, the intensity, coordination, and overall efficiency of this response are far inferior to the highly efficient and stable adaptive state observed in *Lepus yarkandensiss*, which has been consolidated through long-term natural selection.

Abbreviations

The following abbreviations are used in this manuscript:

GFB	Glomerular Filtration Barrier
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
RNA-seq	RNA Sequencing
GSEA	Gene Set Enrichment Analysis
BUN	Blood Urea Nitrogen
Cr	Creatinine
RBC	Red Blood Cell Count
HGB	Hemoglobin Concentration
HCT	Hematocrit
WBC	White Blood Cell Count
UTP	Urine Total Protein
USG	Urine Specific Gravity
Uosm	Urine Osmolality
ADH	Antidiuretic Hormone
HPA	Hypothalamic-Pituitary-Adrenal Axis
ROS	Reactive Oxygen Species
PPP	Pentose Phosphate Pathway

Declarations

AUTHOR CONTRIBUTIONS

Conceptualization, Jianping Zhang and Siquan Liao; methodology, Siquan Liao, Shuai Tian, and Bing Chao; software, Bing Chao; validation, Siquan Liao, Shuai Tian, and Fang Deng; formal analysis, Siquan Liao and Shuai Tian; investigation, Siquan Liao, Fang Deng, and Bu He; resources, Jianping Zhang; data curation, Shuai Tian and Bing Chao; writing—original draft preparation, Siquan Liao; writing—review and editing, Jianping Zhang, Shuai Tian, and Bu He; visualization, Bing Chao; supervision, Jianping Zhang; project administration, Jianping Zhang; funding acquisition, Jianping Zhang. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Animal Ethics and Use Committee of Tarim University (protocol code 2022015).

INFORMED CONSENT STATEMEN

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

References

1. Agaba, E., Rohrscheib, M., & Tzamaloukas, A. (2012). The renal concentrating mechanism and the clinical consequences of its loss. *Nigerian Medical Journal*, *53*(3). https://doi.org/10.4103/0300-1652.104376
2. Coward, R. J. M., Foster, R. R., Patton, D., Ni, L., Lennon, R., Bates, D. O., Harper, S. J., Mathieson, P. W., & Saleem, M. A. (2005). Nephrotic Plasma Alters Slit Diaphragm–Dependent Signaling and Translocates Nephrin, Podocin, and CD2 Associated Protein in Cultured Human Podocytes. *Journal of the American Society of Nephrology*, *16*(3), 629–637. https://doi.org/10.1681/asn.2004030172
3. da Cruz-Filho, J., Costa, D. M., Santos, T. O., da Silva, R. P., Anjos-Santos, H. C., Marciano, N. J. d. S., Rodríguez-Gúzman, R., Henrique-Santos, A. B., Melo, J. E. C., Badauê-Passos, D., Murphy, D., Mecawi, A. S., & Lustrino, D. (2025). Water deprivation induces a systemic procatabolic state that differentially affects oxidative and glycolytic skeletal muscles in male mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *328*(1), R21–R33. https://doi.org/10.1152/ajpregu.00187.2024
4. do Nascimento, N. C., Bailey, T. W., Santos, A. P., Duan, C., Mohallem, R., Franco, J., Aryal, U. K., Xie, J., Cox, A., & Sivasankar, M. P. (2022). Proteomic analysis reveals that aging rabbit vocal folds are more vulnerable to changes caused by systemic dehydration. *BMC Genomics*, *23*(1). https://doi.org/10.1186/s12864-022-08975-x
5. Haraldsson, B., Nyström, J., & Deen, W. M. (2008). Properties of the Glomerular Barrier and Mechanisms of Proteinuria. *Physiological Reviews*, *88*(2), 451–487. https://doi.org/10.1152/physrev.00055.2006
6. Huber, T. B., Hartleben, B., Kim, J., Schmidts, M., Schermer, B., Keil, A., Egger, L., Lecha, R. L., Borner, C., Pavenstädt, H., Shaw, A. S., Walz, G., & Benzing, T. (2023). Nephrin and CD2AP Associate with Phosphoinositide 3-OH Kinase and Stimulate AKT-Dependent Signaling. *Molecular and Cellular Biology*, *23*(14), 4917–4928. https://doi.org/10.1128/mcb.23.14.4917-4928.2003
7. Khegay, I. I. (2023). Reaction of Rat Renal Medullary Laminin to Prolonged Dehydration. *Journal of Evolutionary Biochemistry and Physiology*, *59*(4), 1297–1302. https://doi.org/10.1134/s0022093023040221
8. Li, C., Ruotsalainen, V., Tryggvason, K., Shaw, A. S., & Miner, J. H. (2000). CD2AP is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. *American Journal of Physiology-Renal Physiology*, *279*(4), F785–F792. https://doi.org/10.1152/ajprenal.2000.279.4.F785
9. Liao, S., Peng, D., Chao, B., Deng, F., He, B., & Zhang, J. (2026). Comparative study of renal filtration barrier structure and key gene expression between *Lepus yarkandensis* and Oryctolagus cuniculus. *Experimental Animals*, *75*(3), 306–321. https://doi.org/10.1538/expanim.25-0084
10. Lind, M. I., Yarlett, K., Reger, J., Carter, M. J., & Beckerman, A. P. (2015). The alignment between phenotypic plasticity, the major axis of genetic variation and the response to selection. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1816). https://doi.org/10.1098/rspb.2015.1651

11. Luo, S., Li, Y., Li, S., Jiang, R., Deng, F., Liu, G., & Zhang, J. (2022). Expression Regulation of Water Reabsorption Genes and Transcription Factors in the Kidneys of *Lepus yarkandensis*. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.856427
12. Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N., & Pacheco, M. (2015). Inside the Redbox: Applications of haematology in wildlife monitoring and ecosystem health assessment. *Science of The Total Environment*, *514*, 322–332. https://doi.org/10.1016/j.scitotenv.2015.02.004
13. Mairbäurl, H. (2013). Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Frontiers in Physiology*, *4*. https://doi.org/10.3389/fphys.2013.00332
14. Mbassa, G. K. (1988). Mammalian renal modifications in dry environments. *Veterinary Research Communications*, *12*(1), 1–18. https://doi.org/10.1007/bf00396399
15. Miran, M., Ngo, K., Buob, D., Debiec, H., Ronco, P., & Perry, G. (2025). Microphysiological Glomerular Filtration Barriers: Current Insights, Innovations, and Future Applications. *Advanced Biology*, *9*(9). https://doi.org/10.1002/adbi.202500108
16. Pavenstädt, H., Kriz, W., & Kretzler, M. (2003). Cell Biology of the Glomerular Podocyte. *Physiological Reviews*, *83*(1), 253–307. https://doi.org/10.1152/physrev.00020.2002
17. Pritchard-Jones, K., Fleming, S., Davidson, D., Bickmore, W., Porteous, D., Gosden, C., Bard, J., Buckler, A., Pelletier, J., Housman, D., van Heyningen, V., & Hastie, N. (1990). The candidate Wilms' tumour gene is involved in genitourinary development. *Nature*, *346*(6280), 194–197. https://doi.org/10.1038/346194a0
18. Schwarz, K., Simons, M., Reiser, J., Saleem, M. A., Faul, C., Kriz, W., Shaw, A. S., Holzman, L. B., & Mundel, P. (2001). Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *Journal of Clinical Investigation*, *108*(11), 1621–1629. https://doi.org/10.1172/jci200112849
19. Shih, N.-Y., Li, J., Karpitskii, V., Nguyen, A., Dustin, M. L., Kanagawa, O., Miner, J. H., & Shaw, A. S. (1999). Congenital Nephrotic Syndrome in Mice Lacking CD2-Associated Protein. *Science*, *286*(5438), 312–315. https://doi.org/10.1126/science.286.5438.312
20. Tate, G. J., & Marco, H. G. (2015). Adaptations to life on arid islands of South Africa: renal performance and phenotypic plasticity in the European rabbit Oryctolagus cuniculus. *African Zoology*, *49*(2), 203–212. https://doi.org/10.1080/15627020.2014.11407636
21. Woodard, G. E., Li, X., & Rosado, J. A. (2004). Water deprivation enhances the inhibitory effect of natriuretic peptides on cAMP synthesis in rat renal glomeruli. *American Journal of Physiology-Renal Physiology*, *287*(3), F418–F426. https://doi.org/10.1152/ajprenal.00069.2004
22. Zhang, B., Hu, Y., Jiao, M., Yu, Z., Storey, K. B., Niu, Y., & Wang, H. (2025). Proteomic responses to progressive dehydration and rehydration in Xenopus laevis. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *56*. https://doi.org/10.1016/j.cbd.2025.101654
23. Zhang, J., Li, S., Deng, F., Baikeli, B., Huang, S., Wang, B., & Liu, G. (2019). Higher Expression Levels of Aquaporin Family of Proteins in the Kidneys of Arid-Desert Living *Lepus yarkandensis*. *Frontiers in*

Physiology, *10*. https://doi.org/10.3389/fphys.2019.01172

24. Zhang, J., Li, Y., Shao, D., Chao, B., & Liu, G. (2025). Comparative study of cytochrome P450 expression in the kidneys of *Lepus yarkandensis* and Oryctolagus cuniculus. *Scientific Reports*, *15*(1). https://doi.org/10.1038/s41598-025-91603-x

Figures

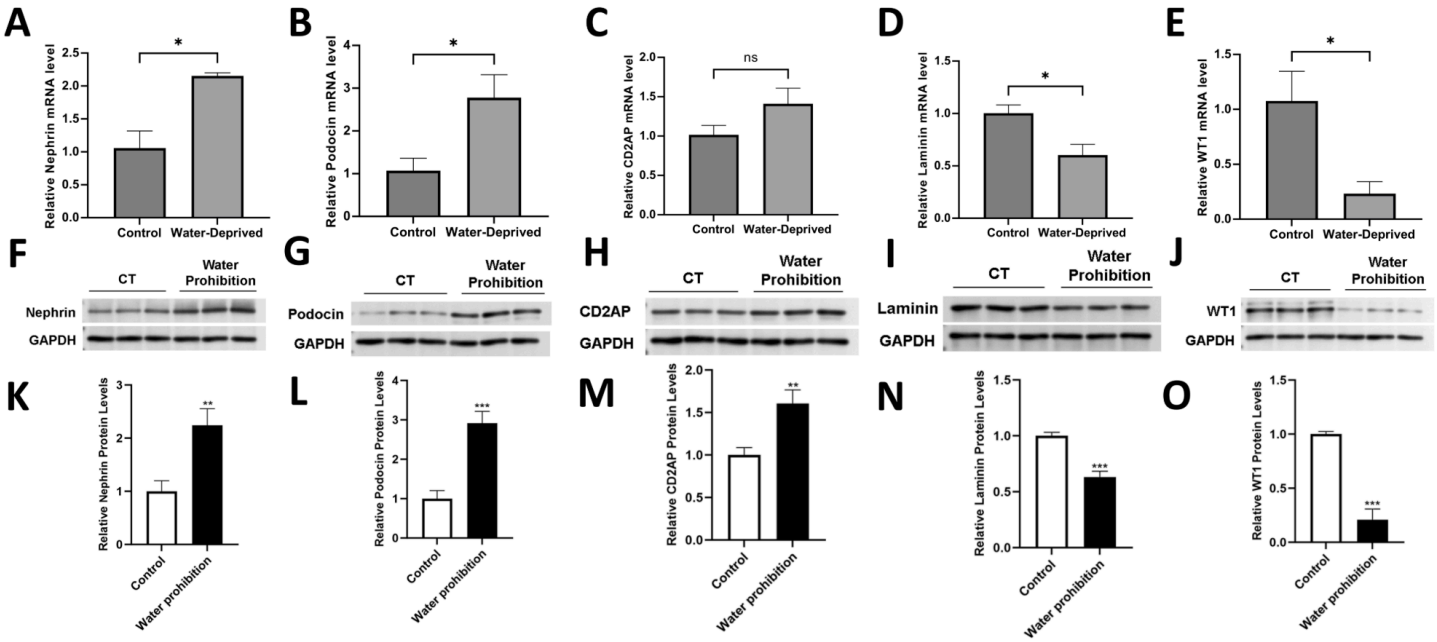


Figure 1

Expression analysis of glomerular filtration barrier-associated genes and proteins in rabbit kidneys under water deprivation stress. (A–E) Quantitative mRNA expression analysis. Relative mRNA levels of Nephrin(A), Podocin(B), CD2AP(C), Laminin(D), and WT1(E) were determined by qRT-PCR and normalized to GAPDH mRNA. Data are expressed as mean ± SD (n ≥ 3). Asterisks indicate statistically significant differences between the Control and Water-Prohibited groups (*p < 0.05, ***p < 0.001; ns, not significant). (F–J) Representative Western blots of protein expression. Immunoblots show the expression of Nephrin (F), Podocin (G), CD2AP (H), Laminin (I), and WT1 (J) proteins, with GAPDH serving as the loading control. CT, control group; Water Prohibition, water-deprived group. Bands are cropped from the same membrane for each target protein. (K–O) Quantitative protein expression analysis. Densitometric analysis of Western blot bands is shown for Nephrin (K), Podocin (L), CD2AP (M), Laminin (N), and WT1 (O). Protein levels were normalized to GAPDH. Data are presented as mean ± SD (n = 3). Statistical significance is indicated as *p < 0.05, p < 0.01, and*p < 0.001 compared to the control group.

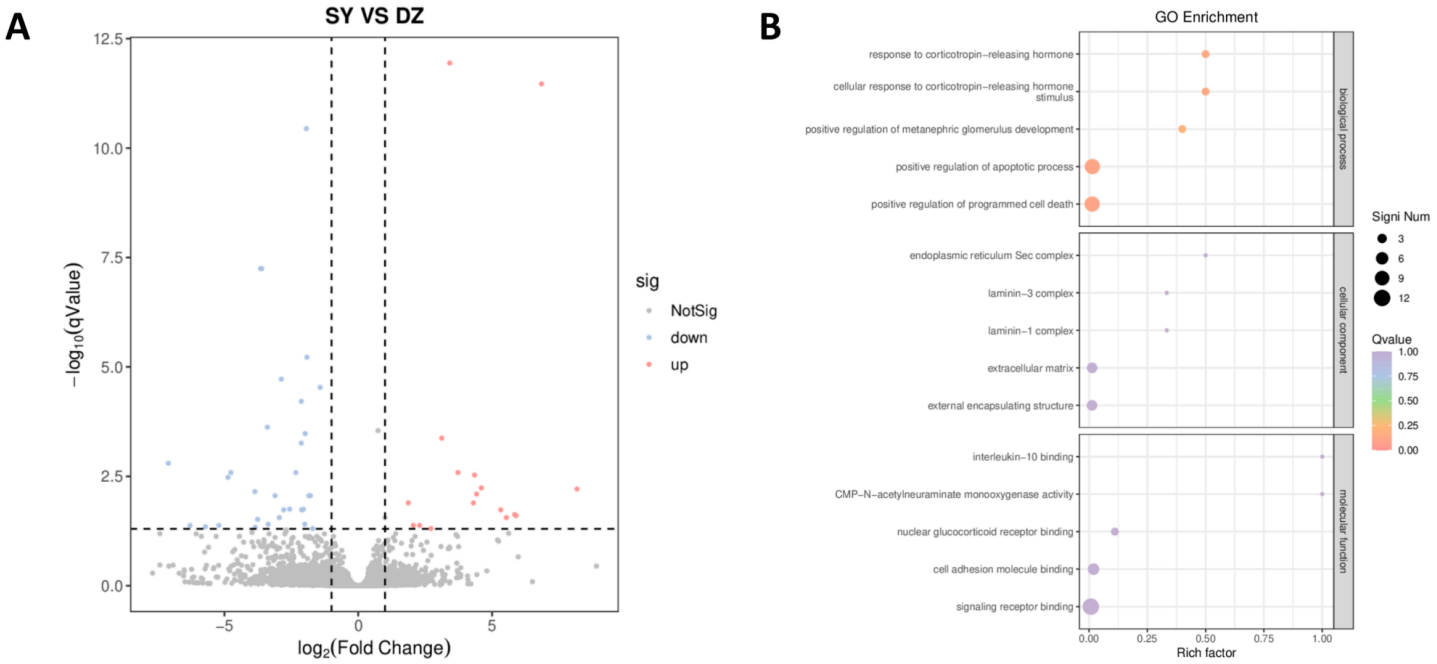


Figure 2

Analysis of differentially expressed genes in rabbit kidney tissue under water deprivation: volcano plot and GO enrichment bubble charts. (A) Volcano plot illustrating the distribution of differentially expressed genes. The x-axis represents the $\log_2$(Fold Change) in gene expression, and the y-axis indicates the statistical significance as $-\log_{10}$(q-value). Genes with significant upregulation, downregulation, or no significant change are denoted in red, blue, and gray, respectively. (B) GO enrichment bubble charts showing the top 10 significantly enriched terms in three ontologies: Biological Process, Cellular Component, and Molecular Function. The size of each bubble corresponds to the number of genes enriched in the term, and the color intensity represents the $-\log_{10}$(q-value), indicating the statistical significance of enrichment. Key terms associated with stress response, apoptosis, extracellular matrix, and hormone receptor binding are highlighted with red borders.

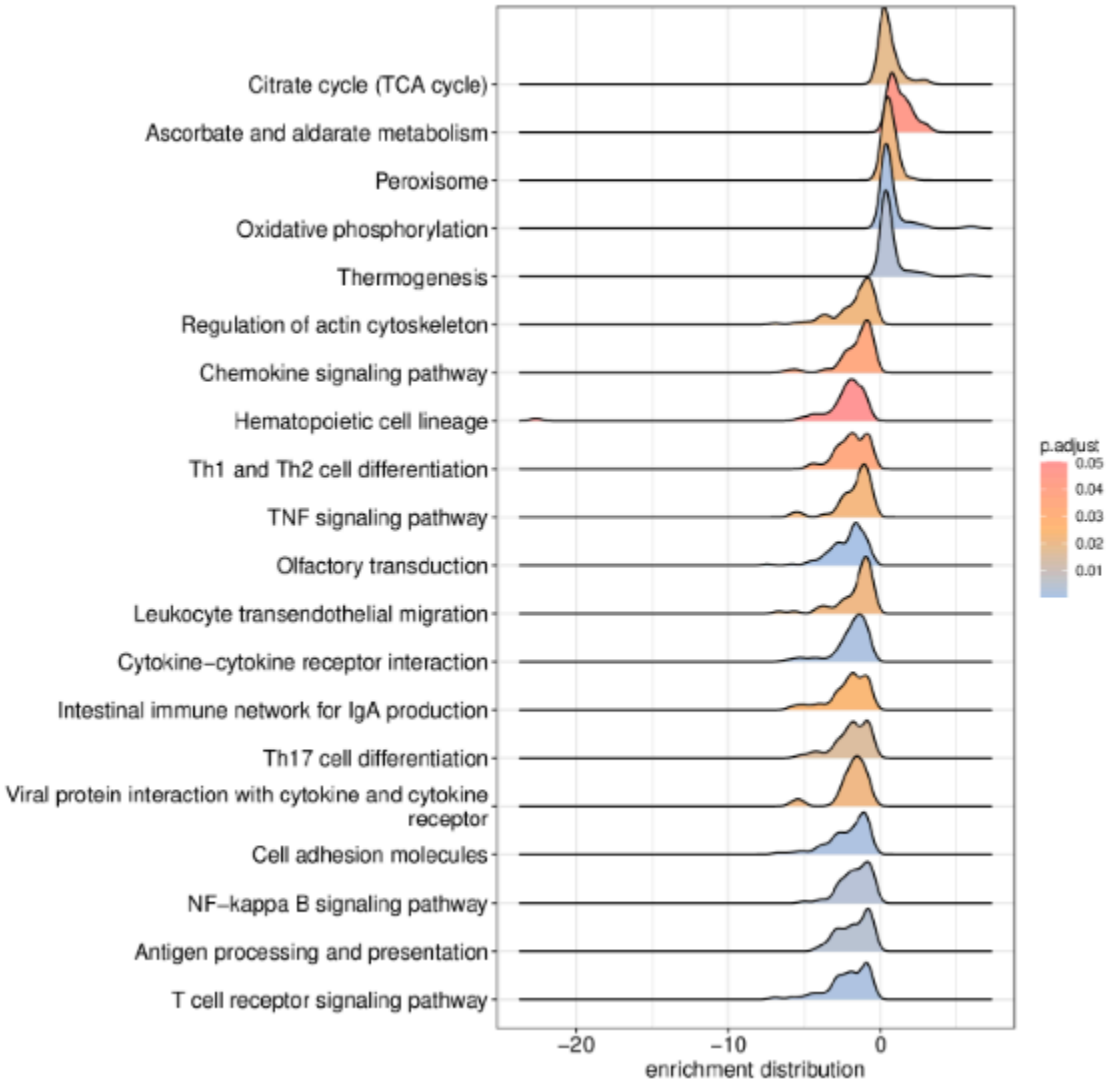


Figure 3

Key pathway enrichment analysis via GSEA in renal tissue of rabbits under water deprivation stress.

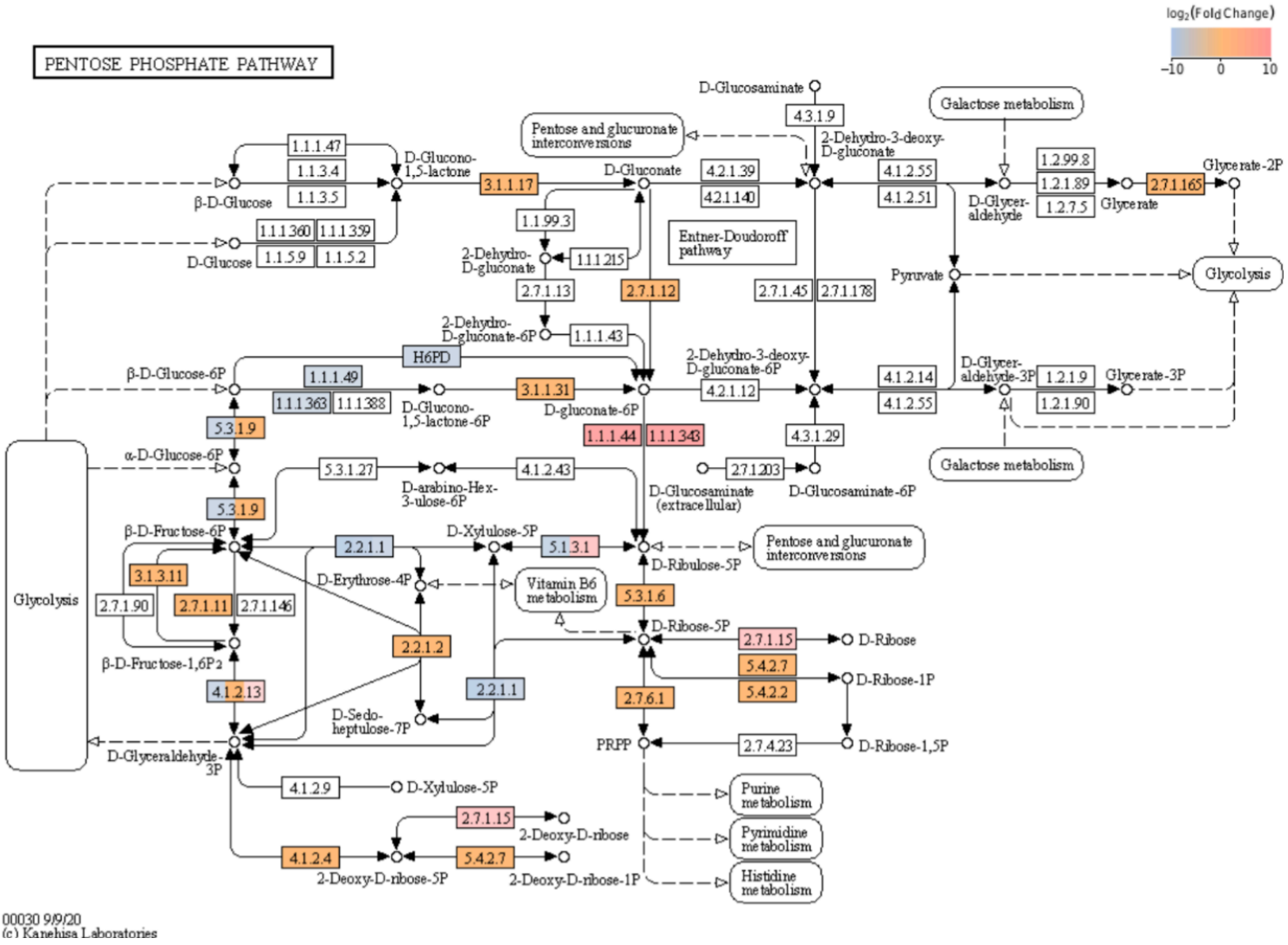


Figure 4

Schematic representation of the significantly enriched pentose phosphate pathway. Each box represents a metabolic enzyme, and the color indicates the differential expression of the corresponding gene. Orange/red represents upregulation, and blue represents downregulation, with the color intensity corresponding to the $\log_2$(Fold Change) scale shown in the upper-right corner.