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ORIGINAL ARTICLE

Effect of hydration on renal activity in a deserticole rodent, Gerbillus tarabuli, subjected to a water-rich diet

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Our work aims to study the effects of hydration (7 days) on the morpho-functional aspect of the kidneys in a rodent deserticola, *Gerbillus tarabuli*, well adapted to water scarcity. Fifteen adult gerbils of mixed-sex are subject to a water-rich diet (15 HG) consisting of lettuce, dates, and barley grains. During seven days, no-hydrated gerbils (15 NHG) receive only barley grains and dates. At the end of the experiment, gerbils were sacrificed, and the kidneys were intended for histological and immunohistochemical study. We examined the renal distribution and expression of aquaporins (AQP1 and AQP2) in the kidneys, and the blood was used for the determination of renal biochemical parameters. Our study's results show that hydration leads to reducing the body weight of gerbils, increasing the relative weights of the kidneys, and decreasing the plasma levels of creatinine, urea, and uric acid at the end of the 7th day. Structurally, the glomeruli have an extension in the glomerular chamber during hydration. *Gerbillus tarabuli* adopt a strategy to conserve body water, and this strategy is essential for low-water hydration; hydration is viewed as a stress factor stimulating the renal activity in order to maintain renal hydromineral balance. **Keywords:** Hydromineral balance, *Gerbillus tarabuli*, Renal activity, Stress, Hydration, Aquaporins.

Introduction

The desert area is characterized by climatic conditions unfavorable to the development of most animals (high temperatures, low and irregular rainfall, etc.) (Haggag and El-Hussein, 1974). Thanks to ecological, anatomical, and physiological adaptation strategies, some desert rodents have acquired a remarkable ability to resist heat and lack of water for long periods (Haggag and El-Hussein, 1974, Degen, 1997; Walsberg, 2000; Bozinovic and Gallardo, 2006).

The previous work has focused on anatomical adaptations. Powell (1987) has showed that desert rodents have a great capacity to absorb water and electrolytes thanks to their small intestine provided with villi and developed brush border. Among these rodents, gerbils do not take free water, and they are limited to the water contained in their nutrition which is produced by metabolic oxidation (Schmidt-Nielsen, 1958; Schmidt-Nielsen, 1964; Ghobrial, 1975; Yagil, 1985). These gerbils have adapted some of their physiological functions and mainly those that contribute to reducing the depletion of water, particularly in the kidneys and the digestive tract. They produce urine concentrated in urea and electrolytes and low-water feces (Schmidt-Nielsen, 1964; Cortes et *al.*, 1988; Palgi and Haim, 2003).

The comparative study of the morphology of the kidney in some desert species has shown certain structural particularities which could reflect an adaptation to their arid environment (Rouffignac et al., 1969; Barrett et al., 1978; Rouffignac et al., 1981, Meng-

Meng and De-Hua, 2016). The kidney of the gerbil, *Gerbillus tarabuli* exhibits a long, narrow renal papilla and low glomerular density, which contributes to the production of concentrated, low-volume urine (Saadi and Lebaili, 2012; Nemiri and Ouali-Hassenaoui, 2020).

Aquaporins (AQPs), a family of membrane proteins which function as water channels, play a crucial role in water reabsorption along the nephron in mammals (Nielsen et al., 2002; King et al., 2004). Among the 8 AQPs distributed in kidney, the knockout of AQP1, AQP2 generates a severe urine-concentrating defect in rats and mice (Verkman, 2009; Kortenoeven and Fenton, 2014).

The physiological mechanism of how gerbils cope with water stress remains unclear. It has been established that the up-regulation of AQP2 expression is a water-sparing mechanism at the molecular level which occurs under conditions of negative water balance. This leads to an increased urine concentrating capacity at the

organismal level (Nielsen et al., 1993; Yang et al., 1999; Nielsen et al., 2002; Bozinovic and Gallardo, 2006). There has been little investigation into the function and regulation of renal AQPs in desert rodents (Bozinovic and Gallardo, 2006), and, consequently, studies on a wider variety of species would further our understanding of this urine-concentrating mechanism (Pannabecker, 2013).

Given the crucial role of the kidneys in the regulation and maintenance of hydromineral balance, we proposed in the present work to seek the involvement of renal activity as a morpho-physiological adaptation strategy in the gerbil, *Gerbillus tarabuli*. This research is based on the study of the repercussions that hydration, through a diet rich in water, can have on the morphological and functional aspect of the kidneys.

Materials and Methods

Animals and experimental design

All animal procedures were licensed and manipulations were performed according to the recommendations of the Algerian ethical committee and under the supervision of authorized investigators.

Thirty adult male and female gerbils (*G. tarabuli*), weighing between 32 g and 53 g, from Algerian arid zones station (Beni Abbes region). These gerbils were divided in to two groups each group consists of gerbils (n=15). After gerbils were acclimatized for 48h prior to the start of the experiment, the animals were housed individually in plastic cages (30 cm x 15 cm x 20 cm) with sawdust as bedding under a constant photoperiod of 14 h and temperature of 24° ± 1° and relative humidity (20-25%).

The firstly group, no-hydrated gerbils (15NHG) were given barley grain (100 g containing: 10.4% water; 9.6% protein; 50.3% sugar; 50.3% cellulose; total mineral content: 2.7%) and dry dates (100 g containing: 15% water; 2.5% protein; 69% sugar; total mineral content: 1.5 to 1.8 g; 7 g fiber) during seven days. The secend group, hydrated gerbils (15HG) were given barley grain; dry dates and fresh lettuce leaves (100 g containing: 95% water; 1.7 g protein; 2% sugar; total mineral content: 2 to 2.6 g: 1% fiber) *ad libitum*, during seven days, Under the rich-water diet, the gerbil orients his dietary choice to wards the lettuce.

Body and relatives kidney weights RKW

The animals were weighed in the morning of the first day and in the end of experiment using a balance (KERN EW420-3NM, Germany). The Kidney were weighed individually with precision balance (KERN ABT 220-5DM, Germany).

Measurement of morphological and physiological parameters

At the end of the experiment, the sacrifices take place in the morning between 9 h and 11 h, the kidneys are removed and intended for a histological and immunohistochemical study, the arteriovenous blood is collected, either on heparin or on EDTA, then centrifuged for 15 min. at 3000 rpm within two minutes. The plasma collected is intended for the determination of creatinine, urea and uric acid.

Immunolocalization of AQPs

The kidneys fixed in Bouin's solution, were paraffin-embedded (7 mm thick). Localization of AQP1 and AQP2 was done by immunohistochemistry following the procedures of (Gallardo et al., 2005). The monoclonal primary antibodies were Mouse anti-

AQP1 (diluted 1: 200, 1/A5F6, Thermo Fisher Scientific (Invitrogen Life Technologies), USA), The polyclonal primary antibodies were Rabbit anti-AQP2 (diluted 1:100, 1/A5F6, Thermo Fisher Scientific (Invitrogen Life Technologies), USA).

Statistical analysis

The results are presented as mean \pm SD. An analysis of the variance and the Student t-test were performed for the comparison of the no-hydrated gerbils group and the hydrated gerbils. The differences are considered significant when *P*<0.05. We will represent three classes of results:

* *P<*0.05

** *P<*0.01

*** *P<*0.001

Results and Discussion

Weight Effects

Effect on body weight: Hydrated gerbils demonstrate a regression in body weight at the end of the 7th day (44.52 \pm 2.01 g) compared to no-hydrated gerbils (40.75 \pm 1.12 g). This difference is statistically insignificant (*P*>0.5) (Fig. 1).



Fig. 1. The average values of body weight of hydrated and no-hydrated adult gerbils. HG: Hydrated Gerbils; D0: Start of the experiment; D7: End of the experiment.

Effect on kidney weight: The absolute weight (182.3 \pm 3.5 mg) and relative kidney weight (446.9 \pm 5.8 mg%) in hydrated gerbils increased compared to absolute weight (172.3 \pm 2.7 mg) and the relative weight (408.5 \pm 9.3 mg%) in no-hydrated gerbils (Fig. 2 and Fig. 3).

The difference is statistically insignificant (P>0.5) in absolute weight and statistically highly significant in relative weight (P<0.01).







Fig. 3. The average values of relative weight of kidney (RKW) of hydrated and no-hydrated adult gerbils. LK: Left Kidney; RK: Right Kidney.

Effects on diuresis and renal plasma parameters

Effects on diuresis: In hydrated gerbils, a statistically highly significant increase (P<0.01) in diuresis (9272.7 ± 71.7 µl/24 h) compared to no-hydrated gerbils (411.1 ± 8.4 µl/24 h) is observed (Fig. 4).



Fig. 4. Influence of hydration on diuresis in no-hydrated and hydrated adult gerbils. HG: hydrated gebils; HNG: no-hydrated gerbils

Effects on plasma kidney parameters

Hydration causes a decrease in plasma creatinine concentration (3.16 \pm 0.18 mg/l) compared to no-hydrated gerbils (4.19 \pm 0.16 mg/l). This decrease is statistically highly significant (*P*<0.01) (Table 1).

A statistically significant (0.05 < P < 0.02) decrease in plasma urea concentration (0.244 ± 0.01 g/l) was observed in hydrated gerbils compared to no-hydrated gerbils (0.281 ± 0.01 g/l) (Table 1).

Plasma uric acid concentration decreased in hydrated gerbils (0.33 \pm 0.08 mg/l) compared to no-hydrated gerbils (2.53 \pm 0.13 mg/l). This decrease is statistically highly significant (*P*<0.01) (Table 1).

Table 1. Plasma Kidney Parameters (Creatinine, Urea and Uric acid) in Gerbillus tarabuli under water-rich diet and dehydration.

Comparative groups	Effectives	Creatinine Pl. (mg/l)	Urea Pl. (g/l)	Uric acid Pl. (mg/l)
No-hyrated Gerbils	15	4.19 ± 0.16	0.281 ± 0.01	2.53 ± 0.13
Hydrated Gerbils	15	3.16 ± 0.18	0.244 ± 0.01	0.33 ± 0.08

Kidney Histology and Immunohistochemistry Kidney histology



Fig. 5. Histology of the renal parenchyma of the hydrated gerbil (A1) and no-hydrated gerbil (A2). Coloration: Hematoxylin and eosin (HE) stain; Magnification: 400 X; BS: Bowman's space; G: Renal Glomerulus; DCT: Distal Convoluted Tube; PCT: Proximal Convoluted Tube; CD: Collecting Duct.



Fig. 6. Histology of the renal parenchyma of the hydrated (B1) gerbil and no-hydrated gerbil (B2). Coloration: Hematoxylin and eosin (HE) stain; Magnification: 1000X; BS: Bowman's space; G: Renal Glomerulus; DCT: Distal Convoluted Tube; PCT: Proximal Convoluted Tube; CD: Collecting Duct.



Fig. 7. Histology of renal medulla of hydrated (C1a,b) gerbil and no-hydrated gerbil (C2a,b). Coloration: Hematoxylin and eosin (HE) stain; Magnification: 400 X; B: Slender Branches; L: Thick ascending limb; CD: Collecting Duct.

- Glomeruli are ovoid and surrounded by a reduced Bowman's space (glomerular chamber) in (HNG) no hydrated Gerbils (Fig. 5 and Fig. 6).
- The analysis of the sections at the level of kidney parenchyma of the hydrated gerbils, the glomeruli demonstrate a remarkable dilation of the glomerular chambers, Bowman's space (Fig. 5 and Fig. 6)
- Most of the tubes surrounding the glomeruli are proximal convoluted tubule (PCT) with a reduced lumen and are more numerous than the distal tubule (DCT) (Fig. 5 and Fig. 6).
- The medulla is taken over mainly by loops of Henlé: the presence of a large number of slender branches and wide ascending branches (Fig. 7).

Immunolocalization and abundance of AQP1, AQP2



Fig. 8. Immunolocalization of aquaporins (AQP1) in the renal cortex of hydrated gerbil (D1) and no hydrated gerbil (D2), *Gerbillus tarabuili*. D1-D2, AQP1 labeling in the proximal tubules; (scale bar=100 µm).



Fig. 9. Immunolocalization of aquaporins (AQP2) in the renal cortex of hydrated gerbil (E1)

and no hydrated gerbil (E2), *Gerbillus tarabuili.* E1-E2, AQP2 labeling in the apical membrane of connecting tubule cells and the cortical collecting duct cells; (scale bar=100 μ m).



Fig. 10. Immunolocalization of aquaporins (AQP1) in the renal medulla of hydrated gerbil (F1) and no hydrated gerbil (F2), *Gerbillus tarabuili*. F1-F2, AQP1 labeling in the descending thin limbs and connecting tubules; (scale bar=100 μm).



Fig. 11. Immunolocalization of aquaporins (AQP2) in the renal medulla of hydrated gerbil (G1) and no hydrated gerbil (G2), *Gerbillus tarabuili*. G1-G2, AQP2 labeling in the apical membrane of collecting duct cells; (scale bar=100 μm).

- Immunostain of AQP1and AQP2 was much lighter in the cortex than the medulla.
- Where we observed the presence of AQP1 in the proximal tubules in the cortex and the descending thin limbs in the medulla.

• AQP2 was more abundant in hydrated gerbils (HG) than no-hydrated gerbils (HNG), and it was correlated by a twice increase in AQP2 abundance in hydrated gerbils (Fig. 8-11).

Discussion

Hydrated gerbils showed a statistically non-significant retrogression in body weight on the seventh day of hydration (Saadi and Lebaili, 2002; Meng-Meng and De-Hua, 2016). This is probably due to the change in feeding behavior by the animal. Gerbils consume lettuce, avoiding barley grains and dates. Contradictory results have been reported by (Saadi, 2001) and (Donath et al., 1999) and Similar results are recorded in the gerbil, *Mongolian gerbils*, by (Meng-Meng and De-Hua, 2016).

Hydration keeps this dimorphism and leads to a statistically non-significant increase in the absolute weight of both kidneys and a statistically highly significant increase in relative weight compared to those of the no hydrated gerbils. This increase may probably reflect the presence of a trophic impact on kidney morphology during hydration. Similar results are recorded in the gerbil, *Gerbillus tarabuli*, by (Zatra, 2008).

Hydrated gerbils showed a considerably higher diuresis rate than no hydrated gerbils. This result reflects the activation of watersaving mechanisms and concentrated urine production in the no hydrated gerbil and the ability to keep low-water metabolism in the hydrated gerbil(Saadi and Lebaili, 2002; Meng-Meng and De-Hua, 2016). (Ben Chaouacha-Chekir, 1989) showed that in desert rodents such as the *Meriones shawi shawi*, the decrease in water metabolism and urine concentration allows them not to modify their volume of body water. The elimination of urine from the kidney depends on the hormones controlling the transport mechanisms at the level of the nephrons, hormones among which vasopressin and aldosterone are the significant contributors (Yagil et al., 1979; Blair-West et al., 1980; De Mccormick et al., 2006).

The activity of gerbils subjected to a water-rich diet showed a decrease in the plasma creatinine, urea, and uric acid concentration compared to that of the no hydrated gerbils. This change is attributed to hemodilution probably accompanied by an increase in renal glomerular filtration and a decrease in tubular reabsorption. These results are similar to those found by (Abdelatif et al., 2010) in which the rate of plasma urea of Namibian goats tends to decrease after 24 hours of rehydration. This decrease is explained by the non-recycling of urea during hydration and the decrease in tubular reabsorption of urea followed by the decrease in circulating vasopressin (Yagil et al., 1979; Blair-West et al., 1980). The latter promotes the reabsorption of urea in the renal tubes.

Creatinine is the best endogenous marker of glomerular filtration and good renal function for a long time (Tsinalis and Binet, 2006). The high concentration of creatinine in no hydrated gerbils could be related to the maintenance of moderate renal function and consequently a reduction in the rate of urine. Creatinine concentration considerably decreased in hydrated gerbils probably reflects the increase of glomerular filtration and urinary excretion to take off the water excess introduced during hydration and adjust the water balance.

The statistically highly significant decrease in uric acid rate in hydrated gerbils can be explained by a large excretion of nitrogen with the urine in response to increased water supply in the body.

The results of renal activity corroborate those of the histological study. According to the analysis of the sections at the level of the kidney parenchyma of the hydrated gerbils, the glomeruli showed a remarkable dilation of the chamber, Bowman's space (Nemiri and Ouali-Hassenaoui, 2020). These results probably confirm the increase in glomerular filtration during hydration to release the water excess provided by the lettuce and to ensure the return of the water balance to a normal state.

AQP1 is the constitutive water-specific channel, and AQP2 is present on the apical membrane in collecting duct cells, where the final fine-tuning of water and urea reabsorption is achieved (Nielsen et al., 2002; Kortenoeven and Fenton, 2014). Similar to other rodents, renal AQP1 abundance of gerbils (*Gerbillus tarabuli*) was unaltered, suggesting that it plays a constitutive role in water absorption (Nielsen et al., 2002).

The up-regulation of renal AQP2 in no-hydrated gerbils is consistent with the leaf-eared mouse following 5 days of water deprivation (Gallardo et al., 2005), free-living degus during summer (Bozinovic et al., 2003), and rats and mice after short periods of water deprivation (Nielsen et al., 1993; Yang et al., 1999). It seems that desert rodents show a generality of AQP1 and AQP2 responses to water deprivations similar to rats and mice, and AQP2 plays an important role of hydromineral balance. AQP1 is present in the proximal tubules in the cortex and the descending thin limbs in the medulla. Neither immunoreactivity nor AQP1 abundance differed between groups (Meng-Meng and De-Hua, 2016).

Conclusion and Perspectives

In its biotope, the gerbil maintains its hydromineral balance by reducing the rate of urinary excretion during water deprivation. This maintenance is ensured by reduced renal activity.

Hydration can be considered as a stress factor engendering a situation of hydromineral imbalance. In response to this situation, renal activity is stimulated: increased filtration and decreased plasma rates of renal parameters see creatinine, urea, and uric acid.

Gerbillus tarabuli showed high flexibility of renal AQPs expression and distribution, to conserve body water, and this flexibility is essential for adaptation and survival in dry and semi-arid desert areas.

The research on the implication of the endocrine and metabolic systems in the body's response to hydration in *Gerbillus tarabuli* could have another way of research.

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