Severe acute dehydration in a desert rodent elicits a transcriptional response that effectively prevents kidney injury

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MacManes MD. Severe acute dehydration in a desert rodent elicits a transcriptional response that effectively prevents kidney injury. Am J Physiol Renal Physiol 313: F262–F272, 2017. First published April 5, 2017; doi:10.1152/ajprenal.00067.2017.—Animals living in desert environments are forced to survive despite severe heat, intense solar radiation, and both acute and chronic dehydration. These animals have evolved phenotypes that effectively address these environmental stressors. To begin to understand the ways in which the desert-adapted rodent Peromyscus eremicus survives, reproductively mature adults were subjected to 72 h of water deprivation, during which they lost, on average, 23% of their body weight. The animals reacted via a series of changes in the kidney, which included modulating expression of genes responsible for reducing the rate of transcription and maintaining water and salt balance. Extracellular matrix turnover appeared to be decreased, and apoptosis was limited. In contrast to the canonical human response, serum creatinine and other biomarkers of kidney injury were not elevated, suggesting that changes in gene expression related to acute dehydration may effectively prohibit widespread kidney damage in the cactus mouse.

Peromyscus; aquaporins; apoptosis; kidney; RNA-Seq

Dehydration, whether caused by exposure to extreme environmental conditions, water deprivation, or infection (e.g., diarrheal illnesses), represents a significant threat to human life (63, 103). In spite of modern medicine, millions of people die every year from dehydration (63). Compounding issues of exposure and illness are public health issues related to the delivery of safe drinking water. With global climate change, it is expected that these challenges will only become more severe (41, 43). As a result, research providing insight into the ecological genomics underlying renal homeostasis and physiological resistance to acute and chronic dehydration is urgently needed. The response to severe acute dehydration in humans and traditional mammalian models is generally limited and may fail to prevent severe electrolyte imbalance, renal impairment or failure, or even death (9, 33, 78, 80). In contrast, desert-living mammals have evolved phenotypes that make them adept at surviving acute and chronic dehydration (67, 93). These phenotypes include numerous aspects of behavior (e.g., nocturnal or crepuscular animals), morphology (e.g., a nasal countercurrent system) (57), physiology (e.g., renal histology) (2, 32, 44, 85), and metabolic water production (15, 93).

In addition to the mechanisms described above, desert animals are thought to possess specialized machinery that allows for the efficient maintenance of water and solute balance in the face of the obvious challenges presented to them as part of desert living. The aquaporins (AQPs), a large protein family integral to the maintenance of water balance in the kidney (11, 54a, 97), were the first genes implicated in desert rodents’ remarkable abilities (39) and immediately became canonized as one of the critical adaptations required by at least some desert rodents. Other studies provided evidence of the importance of this gene family (24, 29, 58, 59), which allows water to be resorbed from the urinary lumen back into the circulation, thereby reducing water loss. Several studies have found members of another gene family, the solute carriers, to be differentially expressed or under lineage-specific positive selection (56, 59). Broadly speaking, these transport molecules are responsible for the maintenance of electrolyte levels required for the normal function of virtually all bodily processes (37). Dehydration and the resultant hyperosmolar state, the direct result of intense heat and aridity, are typically coupled with electrolyte derangement (12, 94); therefore, maintaining the electrolyte gradients required for proper function may be particularly challenging for desert animals. Given these physiological challenges, specialized function of the AQPs and solute carriers may provide at least partial mechanisms, thereby allowing for rodents to survive in desert conditions.

The cell-level consequences of failure of these mechanisms (e.g., salt and water imbalance) are well characterized, at least for non-desert-adapted animals (12, 25, 50). Briefly, dehydration results in a hyperosmotic state, which, among other things, results in cell cycle arrest (64), double-strand DNA breaks (49), disruption of repair mechanisms (19), oxidative stress (101), and inhibition of transcription and translation (77). Additionally, hyperosmolarity is known to inhibit protein folding in the endoplasmic reticulum (ER) (83, 103). Inhibited protein folding results in physical crowding of the ER and invocation of the unfolded protein response, which provides mechanisms for restoring homeostasis, or, in cases where stress is too severe or prolonged, facilitating apoptosis (36). For this reason, the response to a hyperosmolar environment is profound, with disruptions to vital cellular processes. The hypothesis was that these responses will be less profound in an animal adapted to exceptionally dry conditions. Specifically, the prediction was that while cellular stress will be apparent, signs of widespread apoptosis and tissue damage will not. This study seeks to provide transcriptomic evidence to test this hypothesis.

To better understand the effects of dehydration in desert-adapted animals and, in particular, the cellular effects, in this study a set of animals (Peromyscus eremicus) was exposed to...
experimental dehydration. The rodent *P. eremicus* (cactus mouse) represents an ideal model in which to study the genomic architecture of adaptive dehydration resistance and acute dehydration. These animals have evolved in the extremely hot and dry desert regions of the US Southwest (96). They survive their entire lives without drinking water and, in many cases, without urinating. Although highly adapted to desert environments, *P. eremicus* retains many of the useful characteristics of more well-established rodent models, including the facility with which they reproduce in the laboratory (21) and the availability of multiple sequenced genomes (*P. maniculatus*, *P. polionotus*, and *P. californicus*). In addition, the genus *Peromyscus*, known as the *Drosophila* of North American mammalogy (17), has been the focus of ecological study for decades (8, 21, 22, 26, 56, 92) and has recently emerged as a powerful clade of organisms within which to study the genetics of adaptive phenotypes (53, 66, 92).

**METHODS**

**Animal Care and Experimental Model**

The cactus mice used for this study include only captive-born animals purchased from the *Peromyscus* Genetic Stock Center (Columbia, SC). The animals, originally collected from a hot desert location in Arizona, have been housed for several generations at the University of New Hampshire in conditions that mimic temperature and humidity levels in southwestern US deserts, as described previously (47, 48). All animals were fed a standard dehydrated rodent chow (Purina Laboratory Diet Prolab RMH 3000), which contains 22.5% protein, as well as 7.1% fat, 1.63% linolenic acid, and 0.02% arachidonic acid. Animals were randomly assigned to a dehydration treatment group (n = 17, 5 female, 12 male) or a control group (n = 18, 5 female, 13 male). Mice assigned to the dehydration group were weighed and then deprived of water for ~72 h. Mice in the dehydration treatment group were again weighed at the end of the study to estimate dehydration-related weight loss. All mice were then euthanized via isoflurane overdose and decapitation. Trunk blood samples were collected following decapitation for serum electrolyte analyses. Each library was diluted to 2 nM with sterile double-distilled H$_2$O and pooled in a multiplexed library sample, which was sent to the New York Genome Center for 125-bp paired-end sequencing on a HiSeq 2500 platform.

**Illumina Library Preparation and Sequencing**

Tissues frozen in RINaLater were thawed on ice in an RNase-free work environment. Total RNA was extracted through a standard TRIzol extraction protocol (Thermo Fisher Scientific, Waltham, MA) using a <1-mm-thick transverse section that included renal pelvis, medulla, and cortex. The quality of the resultant total RNA extracted was characterized using Tapestation 2200 (Agilent, Santa Clara, CA), after which Illumina sequence libraries were prepared using the TruSeq RNA Stranded LT Kit (Illumina). Tapestation 2200 was used once again to determine the quality and concentration of these libraries. Each library was diluted to 2 nM with sterile double-distilled H$_2$O and pooled in a multiplexed library sample, which was sent to the New York Genome Center for 125-bp paired-end sequencing on a HiSeq 2500 platform.

**Sequence Quality Control and Assembly**

Sequence read data were downloaded, and quality was checked using FastQC version 0.11.5 (3). A de novo transcriptome for the cactus mouse kidney was assembled following the Oyster River Protocol for Transcripome Assembly (55). Briefly, 59 × 10$^6$ paired-end reads from two animals (1 control and 1 dehydrated) were error-corrected using RCorrector version 1.0.2 (91). Adapters, as well as bases, with a Phred score <2 were trimmed using Skewer 0.2.2 (42), and assembly was carried out using Trinity version 2.2.0 (35), Binpacker version 1.0 (54), and Shannon version 0.0.2 (45), and the resultant transcriptomes were merged using the software package transfuse version 5.0.0 (https://github.com/cboursnell/transfuse). Lowly expressed transcripts, defined as those with an abundance of <1 transcript per million (TPM), were filtered out of the data set. The resultant assembly was annotated using the software package dammit (https://github.com/camillescott/dammit) and evaluated using BUSCO 2.0 (88) and TransRate version 1.0.1 (89).

**Mapping and Global Analysis of Differential Gene Expression**

After quality and adapter trimming to a Phred score >2, reads were quasi-mapped to the cactus mouse kidney transcriptome after an index was prepared using Salmon 0.7.2 (73). Quasi-mapping is a newer, yet accepted, technique used by several modern read-mapping software packages that involves leveraging a statistical model, whereby sequencing reads are assigned to their respective transcript of origin, but not actually aligned to that transcript. BLAST was used to map kidney transcripts to genes from the *Mus musculus* proteome version GRCh38.p5 (13). All data were then imported into R statistical package version 3.3.0 (81) using tximport (90) for gene-level evaluation of gene expression, which was calculated using edgeR version 3.1.4 (79) following trimmed mean of M values (TMM) normalization. Specifically, differential gene expression was evaluated using the “glmlRT” test within edgeR, which fits gene-wise negative binomial generalized linear models (GLMs) using gene-specific estimates of dispersion. Uncorrected P values for the two-tailed test are calculated via a likelihood ratio test, which is then corrected for multiple-

| Sample Size | Weight, g | Percent Loss | Water Intake, ml·g$^{-1}·$day$^{-1}$ | Na, mmol/l | Cl, mmol/l | Bicarb, mmol/l | BUN, mg/dl |
|-------------|-----------|--------------|---------------------------------|------------|------------|---------------|------------|
| Dry         |           |              |                                 |            |            |               |            |
| All         | 17        | 21.1         | 22.9                            | 0.1        | 158.7      | 115.5         | 23.2       | 45.6       |
| Female      | 5         | 20.8         | 20.5                            | 0.1        | 157.0      | 115.2         | 22.2       | 41.2       |
| Male        | 12        | 21.1         | 23.5                            | 0.1        | 159.3      | 115.6         | 23.5       | 47.2       |
| Control     |           |              |                                 |            |            |               |            |
| All         | 12        | 20.0         | NA                              | 0.1        | 147.5      | 110.2         | 21.1       | 32.8       |
| Female      | 5         | 20.2         | NA                              | 0.1        | 147.4      | 110.2         | 19.6       | 32.4       |
| Male        | 7         | 19.9         | NA                              | 0.1        | 147.6      | 110.1         | 22.1       | 33.0       |

As reported in Ref. 48, no sex-based differences exist. Note that 6 control animals were sequenced but not included in the physiology study.

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Table 2. Quality-control statistics for the assembled transcriptome: BUSCO

| Score | No. of Contigs | Assembly Size, Mb | Percent Mapping | Percent Bases Uncovered | Percent Contigs Low-Covered |
|-------|----------------|-------------------|----------------|-------------------------|----------------------------|
| PEER Kidney 1.0.0 | 0.31 | 79,660 | 91.7 | 87 | 6 | 8 |

Values are percentages in the cactus mouse kidney transcriptome. Benchmarking universal single-copy orthologs (BUSCO) metrics include statistics regarding the number of universal single-copy orthologs found in the assembly.

Table 3. Quality-control statistics for the assembled transcriptome: TransRate

| Score | No. of Contigs | Assembly Size, Mb | Percent Mapping | Percent Bases Uncovered | Percent Contigs Low-Covered |
|-------|----------------|-------------------|----------------|-------------------------|----------------------------|
| PEER Kidney 1.0.0 | 0.31 | 79,660 | 91.7 | 87 | 6 | 8 |

TransRate metrics are derived from mapping RNA-Seq reads to the assembly, with higher scores indicating a higher-quality assembly. A score of 0.31 ranks this assembly higher than the majority of other published transcriptomes, with 87% of reads mapping, only 6% of bases uncovered (no read support), and 8% of contigs low-covered (mean per-base read coverage <10).

**RESULTS AND DISCUSSION**

**Animal Response to Acute Dehydration**

The response to dehydration is fully described elsewhere (48). Briefly, animals weighed, on average, 20.8 g (n = 29, median = 20.5, SD = 3.9, min = 13.3, max = 32.3) and lost, on average, 22.9% of their body weight (n = 17, median = 23.9%, SD = 5.3%, min = 12.3%, max = 32.3%) after experimental dehydration. The relationship between initial body weight and weight loss is negative but nonsignificant (see Fig. 3 in Ref. 48). Water intake, measured for a subset of the animals are essentially anuric, studies of urine osmolality are not possible.

**Transcriptome Assembly and Evaluation**

The kidney transcriptome assembly consists of 79,660 transcripts. The transcriptome contains 24,794 transcripts with ≥1 hit to the Pfam database, 59,733 hits to OrthoDB (98), 71,857 hits to Uniref90, 2,831 hits to the transporter database (82), and 924 hits to Rfam (34). These 79,660 assembled transcripts map to 13,697 unique genes in the Mus musculus genome. The evaluation using BUSCO and TransRate is presented in Tables 2 and 3, respectively.

**Sequence Read Mapping and Estimation of Gene Expression**

Raw sequencing reads corresponding to individual kidney samples were mapped to the reference kidney transcriptome using Salmon, which resulted in an average mapping rate of 85% (SD = 4%). These mapping data were imported into R and summarized into gene-level counts using tximport; then edgeR was used to generate normalized estimates of gene expression.

**Global Analysis of Differential Gene Expression**

Analysis of global patterns of gene expression, aimed at uncovering previously unknown differences in renal gene expression related to water deprivation by examining the entire transcriptome, was conducted using edgeR. After the count data were normalized using the TMM method (62), which is done by finding a set of scaling factors for the library sizes that minimize the log fold changes between the samples for most genes, we controlled for >13,600 multiple comparisons to yield a robust statistical analysis of differential gene expression. This analysis revealed a dramatic response to acute dehydration in gene expression, with 465 genes (Fig. 1) differentially expressed (252 at a higher level and 213 at a lower level in acute dehydration). Because a transverse section of the kidney was used, the change in expression of any single gene arises as the sum of decreased and/or increased expression in cells located in different regions of the kidney, the function of which is potentially different. All results of the differential expression analysis are available at https://github.com/macmanes-lab/peer_rnaseq/blob/master/all_diff_expression.csv.

GO analysis of the 252 genes more highly expressed in acute dehydration (Table 4) suggests that acute dehydration is linked, first, to the cellular starvation response, which occurs when cells are deprived of critical nutrients, including water. Significantly differentially expressed genes related to this term in-
clude ATF4 (FDR = 0.005, cAMP-dependent transcription factor, known to interact with the ER stress pathway to promote the process of autophagy) (7, 16), SLC2A1 (FDR = 0.003, solute carrier family 2, facilitated glucose transporter), and ATG14 (FDR = 6.3e-05, Beclin 1-associated autophagy-related key regulator), among others. Starvation stress does not seem to invoke widespread apoptosis (see below), which speaks to the ability of these mice to survive dehydration without kidney damage.

Second, genes upregulated in acute dehydration cluster around the GO term related to negative regulation of the MAPK cascade, which is an important regulator of transcription and translation (74), a phenomenon that is known to be a part of the response to hyperosmotic stress (12). Genes involved in this pathway, upregulated in the cactus mouse kidney, include ERRFI1 (FDR = 2.1e-09, ERBB receptor feedback inhibitor 1), XBP1 (FDR = 0.002, X box-binding protein 1), and RGS2 (FDR = 4.6e-07, regulator of G protein signaling 2), the latter of which has been shown to decrease the rate at which water is reabsorbed in the collecting tubules and duct via negative regulation of V2 vasopressin receptor signaling (72, 102).

Lastly, genes upregulated in acute dehydration are related to both positive and negative regulation of apoptosis. When cellular stress is prolonged or intense and prosurvival mechanisms have failed, apoptotic pathways are invoked (27, 46). Genes involved in positive regulation include CTGF (FDR = 3.6e-08, connective tissue growth factor), FOXO3 (FDR = 2.1e-06, forhead box protein O3), CASP8 (FDR = 0.006, caspase-8), and TRP53INP1 (FDR = 0.001, tumor protein p53-inducible nuclear protein 1). In contrast, negative regulation of apoptosis could be important in mitigating the effects of cellular stress in an animal so highly adapted to dehydration. Genes related to this pathway include SOCS3 (FDR = 0.006, suppressor of cytokine signaling 3), CEBPB (FDR = 1.7e-09, CCAAT/enhancer-binding protein-β), PIM3 (FDR = 3.6e-17, serine/threonine-protein kinase pim-3), EDN1 (4.3e-07, endothelin-1), and BCL2L1 (FDR = 1.0e-06, Bcl-2-like protein 1). Indeed, a strong signal of apoptosis is not observed in a global search. For instance, CHOP, the gene that connects the ER stress response to the apoptotic pathways (7, 103), is not differentially expressed (Fig. 2), which suggests that the prosurvival mechanisms are largely successful. Additionally, key genes in the apoptotic pathway (e.g., p53, BIM, BID, BAM, BAK, and CASP2/3) are not differentially expressed, further suggesting that apoptosis is not widespread.

Table 4. Top hierarchical-level GO terms in the biological processes and cellular compartment categories for genes with a higher level of expression in acute dehydration

| Term                                      | No. of Genes | No. of Genes Expected | Fold Enrichment | P Value   |
|-------------------------------------------|--------------|-----------------------|-----------------|-----------|
| **GO biological process**                 |              |                       |                 |           |
| Cellular response to starvation (GO:0009267) | 12           | 1.2                   | 10.2            | 3.4E-05   |
| Positive regulation of apoptotic process (GO:0043065) | 23           | 6.1                   | 3.8             | 5.1E-04   |
| Fatty acid metabolic process (GO:0006631)   | 15           | 3.3                   | 4.5             | 1.3E-02   |
| Regulation of biological quality (GO:0065008) | 63           | 35.2                  | 1.8             | 1.7E-02   |
| Catabolic process (GO:0009056)             | 34           | 14.2                  | 2.4             | 1.8E-02   |
| Negative regulation of apoptotic process (GO:0043066) | 25           | 8.9                   | 2.8             | 3.3E-02   |
| Small molecule biosynthetic process (GO:0044283) | 15           | 3.6                   | 4.1             | 4.2E-02   |
| Negative regulation of MAPK cascade (GO:0043409) | 10           | 1.6                   | 6.3             | 4.8E-02   |
| **GO cellular component**                 |              |                       |                 |           |
| Cytosol (GO:0005829)                       | 41           | 16.1                  | 2.5             | 4.7E-05   |
| Mitochondrion (GO:0005739)                | 38           | 18.5                  | 2.1             | 2.4E-02   |
| Extracellular exosome (GO:00070062)       | 50           | 27.4                  | 1.8             | 2.4E-02   |
| Organelle membrane (GO:0031090)           | 36           | 16.7                  | 2.2             | 1.6E-02   |

GO, gene ontology.
during acute dehydration cluster around the GO term extracellular matrix organization. Genes involved in this pathway that are differentially expressed include fibronectin (FDR = 4.5e-06) and several collagen α-chain genes, as well as lumican (FDR = 0.002; Fig. 3). Fibronectin and collagen have been commonly associated with extracellular matrix turnover and renal interstitial fibrosis (23, 80). Renal interstitial fibrosis, a hallmark of kidney injury, has been causally linked to renal functional impairment (84), with fibrosis leading to a reduction in glomerular filtration rate and overall function. The low level of expression of these genes in the kidney of acutely dehydrated cactus mice provides strong evidence for the hypothesis that dehydration is not related to extracellular matrix turnover and, further, that widespread kidney injury is not occurring.

Critical Process Analysis

To better understand the response to acute dehydration in desert-adapted animals, we analyzed patterns of differential expression between control animals and those exposed to acute dehydration for specific processes proposed to be critical to desert survival. To identify genes related to specific processes of interest (water balance and sodium regulation), we selected relevant GO terms and then identified genes in Mus that correspond to that term. Expression of the Peromyscus orthologs of these genes was estimated.

Water. For desert animals, the regulation of water is obviously critical (15, 67, 85). Here, the AQP genes are thought to be critical to the maintenance of homeostasis and, as such, have been the focus of study for decades (51, 71). How desert animals use these genes to regulate water is largely unstudied, although one study demonstrates that AQP4 is absent in at least one species of Dipodomys rodents (39). The transcriptomic mechanisms used by P. eremicus to regulate water appear to depend on INPP5K (Fig. 4; inositol polyphosphate-5-phosphatase K), which is known to be expressed in the collecting duct, is functionally responsible for urine concentration (75), and is involved in insulin signaling (10). Interestingly, this gene may also have a role in negative regulation of actin in the extracellular matrix (4), which provides yet another indication of the general depression of turnover in the extracellular matrix, as does the lower expression of fibronectin and the collagens.

In addition, AQP4 (Fig. 5) is significantly differentially expressed (FDR = 0.0033): it is lower in acute dehydration.

Table 5. Top hierarchical-level GO terms in the biological processes and cellular compartment categories for genes with a lower level of expression in acute dehydration

| Term                              | No. of Genes | No. of Genes Expected | Fold Enrichment | P Value  |
|-----------------------------------|--------------|-----------------------|-----------------|----------|
| **GO biological process**         |              |                       |                 |          |
| Cholesterol metabolic process     | 15           | 0.9                   | 17.4            | 1.9E-10  |
| Isoprenoid biosynthetic process   | 8            | 0.2                   | 33.1            | 1.6E-06  |
| Extracellular matrix organization | 14           | 1.6                   | 8.8             | 1.0E-05  |
| Positive regulation of bone mineralization | 6        | 0.3                   | 18.8            | 8.4E-03  |
| Cellular response to organic substance | 31          | 12.2                  | 2.5             | 1.5E-02  |
| Response to organonitrogen compound | 17          | 4.6                   | 3.7             | 3.2E-02  |
| Renal system development          | 12           | 2.3                   | 5.2             | 3.8E-02  |
| Response to acid chemical         | 11           | 2.0                   | 5.6             | 5.0E-02  |
| **GO cellular component**         |              |                       |                 |          |
| Extracellular space               | 41           | 13.1                  | 3.1             | 8.4E-08  |
| Fibrillar collagen trimer         | 5            | 0.1                   | 48.2            | 1.1E-04  |
| Endoplasmic reticulum             | 34           | 13.1                  | 2.6             | 4.0E-04  |
| Basement membrane                 | 9            | 0.9                   | 10.1            | 4.7E-04  |
| Cell surface                      | 21           | 7.3                   | 2.9             | 1.8E-02  |
than the control condition. AQP4, expressed in the basolateral membrane of the collecting duct, is responsible for transport of water out of the cell to the interstitium (38, 69). Defects in this non-vasopressin-responsive AQP may result in deficiencies in urine concentration (54b). The functional correlates of lower expression are unknown. On the one hand, lower expression might suggest a reduced capacity for moving water from membrane cells to the interstitium; on the other hand, in severe acute dehydration, very little water is available for movement, so perhaps there is little need to manufacture such protein complexes. No other AQP was significantly differentially expressed. On the one hand, lower expression may not be visible in plots) represent median (filled circle), mean (× and circle), and huber-mu (square). Differential expression was assessed using the global analysis in edgeR. Likelihood ratio test and correction for multiple-hypothesis testing using the FDR method showed statistically significant differential expression of all genes in this plot. FDR values are as follows: 1.717e-05 for COL1A1, 3.235e-10 for COL1A2, 0.0002 for COL2A1, 1.349e-12 for COL3A1 (collagen, type 1, α1), 0.0035 for COL4A1, 0.0033 for COL4A6, 6.608e-09 for COL6A1, 2.111e-09 for COL6A2, 3.608e-15 for COL15A1, 4.451e-06 for FN1 (fibronectin 1), and 0.0018 for LUM (lumican).

**Fig. 3.** Collagen. In all cases, the y-axis is log-transformed TPM. Gray violin plot refers to the acute dehydration group (n = 17); white plot refers to the fully hydrated control animals (n = 18). Violin plot titles are of the format Gene Name:Entrez ID. Symmetrical shape of the plot represents the kernel density estimation of the data. Vertical black line in the middle of the plot represents the range of the data. Darker-gray rectangle portrays 1st and 3rd quartiles of the data. Three lighter-gray symbols (some may not be visible in plots) represent median (filled circle), mean (× and circle), and huber-mu (square). Likelihood ratio test and correction for multiple-hypothesis tests using the FDR method uncovered no significant differential expression.

**Fig. 4.** Regulation of renal water transport. In all cases, the y-axis is log-transformed TPM. Gray violin plot refers to the acute dehydration group (n = 17); white plot refers to the fully hydrated control animals (n = 18). Violin plot titles are of the format Gene Name:Entrez ID. Symmetrical shape of the plot represents the kernel density estimation of the data. Vertical black line in the middle of the plot represents the range of the data. Darker-gray rectangle portrays 1st and 3rd quartiles of the data. Three lighter-gray symbols (some may not be visible in plots) represent median (filled circle), mean (× and circle), and huber-mu (square). Likelihood ratio test and correction for multiple-hypothesis tests using the FDR method uncovered no significant differential expression.

**Sodium.** In addition to management of water, desert animals must also manage the maintenance of salts, namely, sodium. Indeed, hypernatremia is common in dehydration (94) and is observed in acutely dehydrated cactus mice (48). In response to dehydration-related hypernatremia, significant differential expression is detected (Fig. 6) in AVPR2 (FDR = 0.0066; arginine vasopressin receptor 2), SLC8A1 (FDR = 4.451e-06; solute carrier family 8 member A1), SCNN1A (FDR = 0.0008; epithelial sodium channel subunit 1α), AGT (FDR = 0.0004; angiotensinogen), and REN (FDR = 0.0046; renin), with AVPR2 and SLC8A1 being more highly expressed in control animals. AVPR2, a vasopressin receptor, has been linked to both sodium excretion (5) and development of nephrogenic diabetes insipidus (68), while SLC8A1 is a sodium/calcium exchanger (28). Highly expressed in acute dehydration, SCNN1A is one component of the renal epithelial sodium channel (14) and has been linked to the development of pseudohypoaldosteronism (18, 99), a condition in which sodium is released inappropriately, resulting in hyponatremia. Lastly, AGT and REN, more highly expressed in acute dehydration, are thought to be broadly involved in the management of blood pressure and sodium excretion (30, 40). In summary and in contrast to the relatively simple mechanisms through which water is regulated, the maintenance of serum sodium levels is seemingly more complex, with multiple genes working in concert, in the face of hypernatremia, to regulate serum sodium. Elucidation of the individual roles of each gene uncovered here could be an exceptionally fruitful area of research. For instance, does each gene have a specific role in maintaining balance, or is there a high degree of redundancy in the regulation of serum sodium? Alternatively, does each region of the kidney (e.g., cortex, medulla, and specific parts of the nephron) employ different genes, each uniquely specialized? Future research employing spatially explicit RNA-Seq or

**Fig. 6.** Violin plot portrays 1st and 3rd quartiles of the data. Three lighter-gray symbols (some may not be visible in plots) represent median (filled circle), mean (× and circle), and huber-mu (square). Differential expression was assessed using the global analysis in edgeR. Likelihood ratio test and correction for multiple-hypothesis testing using the FDR method showed statistically significant differential expression of all genes in this plot. FDR values are as follows: 1.717e-05 for COL1A1, 3.235e-10 for COL1A2, 0.0002 for COL2A1, 1.349e-12 for COL3A1 (collagen, type 1, α1), 0.0035 for COL4A1, 0.0033 for COL4A6, 6.608e-09 for COL6A1, 2.111e-09 for COL6A2, 3.608e-15 for COL15A1, 4.451e-06 for FN1 (fibronectin 1), and 0.0018 for LUM (lumican).
single-cell genomic techniques could offer additional insights into the molecular genetics of renal sodium regulation.

**Coexpression Network Analysis**

WCGNA analysis identified 22 coexpression modules related to the measured phenotypes. While nearly all modules had significant relationships with at least one physiological measurement (Fig. 7), several modules showed a particularly tight linkage with physiology. The GO terms of each module are available at https://github.com/macmanes-lab/peer_rnaseq/blob/master/GOEnrichmentTable.csv. The dehydration-linked weight loss of animals has a significant positive (higher gene expression related to more weight loss) relationship with the “tan” module, which contains the GO terms transmembrane signaling receptor activity, circulatory and cardiovascular system development, and peptide hormone binding. The three described genes most tightly linked to this module include CPM (carboxypeptidase M), which potentially has an angiotensin-converting function (20), SDK2 (sidekick cell adhesion molecule 2), and GALC (galactosylceramidase). Dehydration-linked weight loss of animals has a significant negative (higher gene expression related to less weight loss) relationship with the “gray” module, which contains GO terms linked to monooxygenase activity, the steroid metabolic process, and negative regulation of transport. The three described genes most tightly linked to this module include ITGA9 (integrin-α9), SGCG (sarcoglycan-γ), and MAGB4 (melanoma antigen, family B, 4). Broadly speaking, the integrins are receptors for extracellular matrix components (76) and are involved in the regulation of intracellular osmolarity (65). Expression of integrins, which could allow for sensing of osmotic stress and subsequent production of osmolytes, seems to have important links to dehydration-linked weight loss.

Sodium and chloride ion concentrations were positively associated (higher gene expression related to higher serum concentrations) with genes in the “royal blue” module, which contained the GO terms vitamin transport, water transmembrane transporter activity, and water channel activity. Top genes in this group include CYP4B1 (cytochrome P-450, family 4, subfamily b), GSTA1 (glutathione S-transferase-α1),

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**Fig. 5.** Cellular water homeostasis. In all cases, the y-axis is log-transformed TPM. Gray violin plot refers to the acute dehydration group (n=17); white plot refers to the fully hydrated control animals (n=18). Violin plot titles are of the format Gene Name:Entrez ID. Symmetrical shape of the plot represents the kernel density estimation of the data. Vertical black line in the middle of the plot represents the range of the data. Darker-gray rectangle portrays 1st and 3rd quartiles of the data. Three lighter-gray symbols (some may not be visible in plots) represent the median (filled circle), mean (× and circle), and huber-mu (square). Differential expression was assessed using the global analysis in edgeR. Likelihood ratio test and correction for multiple-hypothesis tests using the FDR method show significantly differential expression of aquaporin (AQP) 4 (FDR = 0.0033).

**Fig. 6.** Sodium ion homeostasis. In all cases, the y-axis is log-transformed TPM. Gray violin plot refers to the acute dehydration group (n=17); white plot refers to the fully hydrated control animals (n=18). Violin plot titles are of the format Gene Name:Entrez ID. Symmetrical shape of the plot represents the kernel density estimation of the data. Vertical black line in the middle of the plot represents the range of the data. Darker-gray rectangle portrays 1st and 3rd quartile of the data. Three lighter-gray symbols (some may not be visible in plots) represent the median (filled circle), mean (× and circle), and huber-mu (square). Likelihood ratio test and correction for multiple-hypothesis tests using the FDR method show significantly differential expression of AVPR2, 4.4512e-06 for SLC8A1, 0.0008 for SCNN1A, 0.0004 for AGT, and 0.0046 for REN.
and LTA4H (leukotriene A4 hydrolase). Sodium and chloride ion concentration has a negative relationship with genes in the gray module (see dehydration-linked weight loss), suggesting the tight physiological link between water and electrolyte homeostasis.

### Biomarkers of Kidney Damage

A great deal of research has been done to identify markers of acute kidney injury (31, 70, 86, 103), and, as a result, the measurement of kidney damage has moved well beyond assay

**Fig. 7. Heat map describing the trait-module relationship in the data set. Left axis: arbitrarily assigned colors representing the co-expression module; right axis, strength of the correlation. Values are correlation coefficients, with $P$ value of the correlation in parentheses. BUN, blood urea nitrogen; Cr, creatinine.**

**Fig. 8. Biomarkers of acute kidney injury. In all cases, the y-axis is log-transformed TPM. Gray violin plot refers to the acute dehydration group ($n = 17$); white plot refers to the fully hydrated control animals ($n = 18$). Violin plot titles are of the format Gene Name:Entrez ID. Symmetrical shape of the plot represents the kernel density estimation of the data. Vertical black line in the middle of the plot represents the range of the data. Darker-gray rectangle portrays 1st and 3rd quartiles of the data. Three lighter-gray symbols (some may not be visible in plots) represent the median (filled circle), mean ($\mu$ and circle), and huber-$\mu$ (square). Differential expression was assessed using the global analysis in edgeR. Likelihood ratio test and correction for multiple-hypothesis tests using the FDR method uncovered no significant differential expression.**
of serum creatinine, which is known to be relatively insensitive and slow to indicate damage (6). In humans, dehydration and other clinical conditions that cause a pathological reduction in renal perfusion pressure are responsible for acute kidney injury (95). If acute dehydration results in substantial tissue damage in the cactus mouse, this damage will be evident in a panel of biomarkers specific for kidney injury. Despite these markers not having been validated in the cactus mouse, the fact that a large number of statistically independent tests are concordant (Fig. 8), showing no differential expression, suggests that no significant tissue damage is occurring.

Conclusion

Animals living in desert environments are forced to survive despite severe heat, intense solar radiation, and both acute and chronic dehydration. Indeed, these animals have evolved phenotypes that address these environmental stressors. To begin to understand the ways in which the desert-adapted rodent Perognathus eremicus survives, reproductively mature adults were subjected to profound acute dehydration, during which they lost, on average, 23% of their body weight over the 3-day experiment. We modeled this loss after a scenario of a summertime rainfall event followed by intense drought. While animals lost a substantial amount of weight, they were behaviorally intact; i.e., qualitatively, they continued to be active, eat, and interact normally with caregivers and, when possible, conspecs. Animals reacted via a series of changes in the kidney, which included upregulation of genes responsible for 1) reducing the rate of transcription and 2) maintaining water and salt balance. Extracellular matrix turnover appeared to be substantially decreased, and apoptosis appeared to be limited. Serum creatinine and other markers of kidney injury were not elevated over baseline, which was different from Mus exposed to less severe dehydration (80), suggesting that acute dehydration was not responsible for widespread kidney damage in the cactus mouse.

The work presented here represents results of an experiment allowing an in-depth examination into the renal machinery of Perognathus eremicus, allowing for survival despite severe acute dehydration. In contrast, humans are exquisitely sensitive to alteration of water balance, with even minor dehydration resulting in physiological compromise (94). The molecular toolkit employed by the cactus mouse, i.e., the genes identified in this study, have orthologs in humans. One exciting potential outcome of this line of work is the ability to develop manipulative genetic or pharmacological techniques to enable a more robust human response to dehydration. Such a response could be extremely valuable in a wide variety of situations where potable water is limited.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

M.D.M. conceived and designed research; M.D.M. performed experiments; M.D.M. analyzed data; M.D.M. interpreted results of experiments; M.D.M. prepared figures; M.D.M. drafted manuscript; M.D.M. edited and revised manuscript; M.D.M. approved final version of manuscript.

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