

1 **Physiological and biochemical changes associated with acute experimental**  
2 **dehydration in the desert adapted mouse, *Peromyscus eremicus***

3  
4 Lauren Kordonowy<sup>1</sup>, Kaelina D. Lombardo<sup>1</sup>, Hannah L. Green<sup>2</sup>, Molly Dawson<sup>2</sup>, Evice  
5 A. Bolton<sup>1</sup>, Sarah LaCourse<sup>3</sup>, Matthew D. MacManes<sup>1\*</sup>

6  
7 <sup>1</sup> University of New Hampshire, Department of Molecular Cellular and Biomedical  
8 Sciences

9 <sup>2</sup> University of New Hampshire, Department of Biological Sciences

10 <sup>3</sup> University of New Hampshire, Department of Psychology

11 \* [matthew.macmanes@unh.edu](mailto:matthew.macmanes@unh.edu)

12

13 Running title: Electrolyte change in acute dehydration

14

15 Keywords: Dehydration, desert, electrolyte, rodent

16

17 Summary statement: The establishment of baseline values for serum electrolytes  
18 and water intake, as well as their response to acute dehydration is critical  
19 for characterizing the physiology necessary for desert survival.

20

21 Conflict of Interest Statement: The authors declare no conflict of interest.

22

23

24

25

26

27

28

29

30

31

32

33

34 **Abstract**

35 Characterizing traits critical for adaptation to a given environment is an  
36 important first step in understanding how phenotypes evolve. How animals adapt  
37 to the extreme heat and aridity commonplace to deserts represents is an  
38 exceptionally interesting example of these processes, and has been the focus  
39 of study for decades. In contrast to those studies, where experiments are  
40 conducted on either wild animals or captive animals held in non-desert  
41 conditions, the study described here leverages a unique environmental chamber  
42 that replicates desert conditions for captive *Peromyscus eremicus* (cactus  
43 mouse). Here we establish baseline values for daily water intake and for serum  
44 electrolytes, as well as the response of these variables to experimental  
45 dehydration. In brief, *P. eremicus*' daily water intake is very low. It's serum  
46 electrolytes are distinct from many previously studied animals, and its  
47 response to acute dehydration is profound, though not suggestive of renal  
48 impairment, which is atypical of mammals.

49

50

51 **Introduction**

52 Understanding the evolution of adaptive traits has long been one of the primary  
53 goals in evolutionary biology. The study of the relationships between fitness  
54 and phenotype, often powered by modern genomic techniques (59), has provided  
55 researchers with insight into the mechanistic processes that underlie adaptive  
56 phenotypes (15, 28). Systems in which the power of genomics can be combined  
57 with an understanding of natural history and physiology are well suited for  
58 the study of adaptation (9, 44) especially when researchers have the ability  
59 to assay the link between genotype and phenotype in wild animals and then  
60 conduct complementary experiments using representative animals in carefully  
61 controlled laboratory environments. The study described here, characterizing  
62 the physiology and serum biochemistry of *Peromyscus eremicus* is the first step  
63 in a larger study aimed at understanding the genomics architecture of adaptation  
64 to desert environments.

65

66 Desert adaptation has significant ecological, evolutionary, and biomedical  
67 significance. In contrast to humans and other mammals, desert rodents can  
68 survive in extreme environmental conditions and are resistant to the effects  
69 of dehydration. Physiological adaptations to deserts have been characterized in  
70 several rodents. Specifically, renal histology has been studied in multiple  
71 Heteromyid rodents (3), and the general conclusion is that these desert adapted  
72 animals have evolved elongate Loops of Henle (7, 10, 38) that are hypothesized  
73 to optimize water conservation. In addition to studies of renal histology,  
74 several studies have characterized pulmonary water loss (23, 51), water  
75 metabolism (26), and water consumption (12, 34, 35, 41, 46) in desert rodents.  
76 While desert animals possess specialized physiology that is efficient with  
77 regards to water metabolism and loss, whether or not specialized genomic  
78 adaptation exists is an active area of research (32, 36, 37).

79

80 Although the cactus mouse (*Peromyscus eremicus*) has not been a particular focus  
81 for the study of desert adaptation (but see (2, 32), this Cricetid rodent  
82 native to the arid regions of the Southwestern United States and Northern  
83 Mexico (57) offers a unique opportunity to understand physiological adaptations  
84 to deserts. *P. eremicus* is a member of a larger genus of animals known  
85 colloquially as the “*Drosophila* of mammals” (9), and *Peromyscus* species have  
86 been the focus of extensive study (25, 33, 52, 54). *P. eremicus* is a sister  
87 species to the non-desert adapted *P. californicus* (13), and it is closely  
88 related to *P. crinitus*, the canyon mouse, which is another desert adapted  
89 rodent native to Southwestern deserts.

90

91 Critical to desert survival is the ability to maintain water balance even when  
92 the acute loss of water exceeds dietary water intake (24). Indeed, the mammalian  
93 corpus consists of 60% water (30). Far from a static reservoir, proper  
94 physiologic function requires water for numerous processes, including nutrient  
95 transport (22), signal transduction, pH balance, thermal regulation (42) and  
96 the removal of metabolic waste. To accomplish these functions, a nearly constant  
97 supply of water is required to replace water loss (30), which occurs mainly  
98 via the gastrointestinal and genitourinary systems, and evaporative loss, which

99 is greatly accelerated in extreme heat and aridity (16). Because the body  
100 possesses limited reserves, when loss exceeds intake during even a short period  
101 of time, dehydration and death can occur. Mammals are exquisitely sensitive to  
102 dehydration and possess limited compensatory mechanisms.

103

104 Characterizing desert adaptation requires careful and integrative physiological  
105 studies, which should include a detailed characterization of water intake,  
106 responses to dehydration, and the measurement of blood electrolytes. Indeed,  
107 quantifying these metrics is one of the first steps in understanding how animals  
108 survive in the extreme heat and aridity of deserts. In particular, the  
109 electrolytes chloride and sodium are important markers of dehydration (18).  
110 These molecules play essential roles in metabolic and physiological processes,  
111 and they are integral to the functionality of a variety of transmembrane  
112 transport pumps (11, 29), neurotransmission (62), and maintenance of tonicity  
113 (19). Furthermore, hyponatremia causes restlessness, lethargy, muscle weakness,  
114 or coma (1). Bicarbonate ion, in contrast, is primarily responsible for aiding  
115 in the maintenance of the acid-base balance and is resorbed in the renal tubules  
116 (39). Blood urea nitrogen (BUN) is a test that assays the abundance of urea -  
117 the end-product for metabolism of nitrogen containing compounds. Urea is  
118 resorbed in the glomerulus, and renal impairment is often inferred when BUN  
119 becomes elevated (8). Importantly, the canonical model of urea resorption is  
120 dependent on urine volume, which is markedly diminished in desert rodents, thus  
121 limiting the utility of using BUN as an indicator of renal function. Lastly,  
122 creatinine, a product of muscle breakdown, whose measured level does not depend  
123 on urine volume is used as a measure of renal function (8).

124

125 Genes most frequently implicated in desert-adaptation include members of the  
126 aquaporin family (27). However, previous work suggests that an alternative  
127 gene family, the solute carriers, are more relevant for desert-adaptation in  
128 the cactus mouse (32). As a first step towards fully elucidating the patterns  
129 of adaptive evolution to deserts in *P. eremicus*, we characterized the normal  
130 patterns of water intake and electrolyte levels as well as the physiologic  
131 response to experimental dehydration. As such, this study provides critical

132 physiological and biochemical information about *P. eremicus* and its response  
133 to dehydration and is generally useful as researchers begin to leverage large-  
134 scale genome data against classic questions regarding the evolution of adaptive  
135 phenotypes.

136

### 137 **Materials and Methods**

138 We used captive *P. eremicus* (n=44, 24 male, 20 female) that were descendant  
139 from mice purchased from the University of South Carolina Peromyscus Genetic  
140 Stock Center. The USC colony was founded using wild caught animals from a dry-  
141 desert population in Arizona. For ongoing experimental purposes, animals are  
142 housed in a large walk-in environmental chamber built to replicate the  
143 environmental conditions in which this population has evolved. Specifically,  
144 the animals experience a normal diurnal pattern of temperature fluctuation,  
145 ranging from 90F during the daytime to 75F during the night. Relative humidity  
146 (RH) ranges from 10% during the day to 25% during the night. Animals are housed  
147 in standard lab mouse cages with bedding that has been dehydrated to match  
148 desert conditions. They are fed a standard rodent chow, which has also been  
149 dehydrated. Water is provided *ad Lib* during certain phases of experimentation  
150 and withheld completely during others. All animal care procedures follow the  
151 guidelines established by the American Society of Mammalogy (53) and have been  
152 approved by the University of New Hampshire Animal Care and Use Committee under  
153 protocol number 103092.

154

155 All animals included in this study were sexually mature adults, as defined for  
156 males as having scrotal testes and for females as having a perforate vaginal  
157 meatus. A slight bias for the inclusion of males exists, as a concurrent study  
158 of male reproductive genomics was occurring. Preliminary analyses conducted  
159 suggest that no significant differences in any of the physiological measures,  
160 and thus, males and females were analyzed as one group. For a subset of animals,  
161 water intake was measured, which was accomplished via the use of customized  
162 15ml conical tubes, wherein water intake was measured every 24 hours for a  
163 minimum of 3 consecutive days (range 3-10 days). Animals selected for the  
164 dehydration trial were weighed on a digital scale, housed without water for

165 three days, then re-weighed to determine the change in body mass due to  
166 dehydration. At the conclusion of water measurement or after a three-day  
167 dehydration animals were sacrificed via isoflurane overdose and decapitation.  
168 Immediately after death, a 120uL sample of trunk blood was obtained for serum  
169 electrolyte measurement. This was accomplished using an Abaxis Vetscan VS2  
170 machine with a critical care cartridge, which measures the concentration of  
171 several electrolytes (Sodium, Chloride, Bicarbonate ion, Creatinine, and Blood  
172 Urea Nitrogen (BUN)) relevant to hydration status and renal function. Lastly,  
173 the kidney, spleen, liver, lung, hypothalamus, testes, vas deferens and  
174 epididymis were dissected out and stored in RNAlater (Ambion Inc.) for future  
175 study. All statistical analyses were carried out in the statistical package,  
176 R (50).

177

## 178 **Results**

179 We measured the daily water intake for 44 adult cactus mice (24 male, 20 female)  
180 for between three and ten consecutive days. Mean water intake was 0.11 mL per  
181 day per gram body weight (median=0.11, SD=0.05, min=0.033, max=0.23). We  
182 measured levels of serum Sodium, Chloride, Bicarbonate ion, Creatinine, and  
183 Blood Urea Nitrogen (BUN) for the same 44 adult mice, thereby establishing  
184 normal (baseline) values for *P. eremicus* (Figure 1 and Table 1).

185

186 A comparison of mice provided with water *ad libitum* to mice that exposed to  
187 experimental water deprivation for three days revealed that the dehydrated mice  
188 lost an average of 23.2% of their body weight (median=23.9%, SD=5.3%, min=12.3%,  
189 max=32.3%, n=13 dehydration treatment, 7 males, 6 females). Despite this  
190 substantial weight loss, anecdotally, mice appeared healthy. They were active,  
191 eating, and interacting with handlers and other mice, normally. The amount of  
192 weight loss did not depend on daily water intake ( $p=0.63$ ,  $R^2=0.03$ ), though the  
193 trend suggests that animals that drink more water lost more weight). Furthermore,  
194 body weight did not strongly influence the percent loss of body weight (Figure  
195 2;  $p=0.68$ ,  $R^2=0.02$ ).

196

197 In addition to a substantial loss in body weight, dehydration was associated  
198 with differences in serum electrolytes (Figure 3; n=13 dehydrated, n=31  
199 hydrated). These changes were subtle, but significant using a two-sample t-  
200 test ( $p < 0.008$  in all cases).

201  
202 Lastly, the levels of serum electrolytes were tightly correlated with percent  
203 body weight loss (Figure 4). Indeed, the relationship between the level of  
204 serum sodium and weight loss was positive and significant, (ANOVA, F-statistic:  
205 12.85, 11 DF,  $p = 0.004$ ), as was the relationship between BUN and weight loss  
206 (ANOVA, F-statistic: 9.089, 11 DF,  $p = 0.012$ ). The relationships between weight  
207 loss and chloride and bicarbonate levels respectively, were positive but not  
208 significant. Of note, all data will be deposited in Dryad upon acceptance for  
209 publication.

210

## 211 **Discussion**

212 Deserts are amongst the harshest environments on the planet. Indeed, animals  
213 living in these areas must be highly adapted to the unique combination of  
214 extreme heat and aridity. Given that our understanding of the physiology of  
215 desert adapted animals is limited largely to studies in renal histology (38)  
216 and on water intake and output (34, 56), an enhanced understanding of serum  
217 electrolyte changes due to dehydration is informative. Because many of the  
218 harmful effects of dehydration result from electrolyte abnormalities,  
219 characterizing normal values and the electrolyte response to dehydration  
220 represents a critical first step in garnering a deeper understanding of how  
221 desert animals survive despite severe and prolonged dehydration.

222

223 In this study, normal (baseline) values for serum Sodium, Chloride, Bicarbonate  
224 Ion, Creatinine, and Blood Urea Nitrogen were established in a captive colony  
225 of lab animals housed in desert conditions. Although these measures may differ  
226 in wild animals (see (14) for a brief review of such differences), establishing  
227 normal values in captive animals is crucial, though future studies aim to  
228 understand the patterns of electrolyte variation in wild animals. In *P. eremicus*,  
229 we define the normal ranges for each electrolyte as those values falling between

230 the 1<sup>st</sup> and 3<sup>rd</sup> quartile. Serum Chloride and Sodium were significantly higher  
231 than in published ranges for other mammals, including humans, a marsupial (58),  
232 *Cricetomys* (48), and the porcupine (43). However, serum chloride and sodium  
233 levels in our study were quite comparable to another wild rodent, *Neotoma*  
234 *fuscipes* (61), a Mustelid (55), and the Hyrax (5). Values for BUN are generally  
235 higher in this study; unfortunately, a direct comparison is not possible, as  
236 measured values are dependent on the volume of urine produced. Serum Creatinine  
237 is low, largely resulting from the general lack of muscle mass in *P. eremicus*  
238 relative to other mammals. However, because the equipment used to analyze this  
239 electrolyte does not effectively capture the lower end of the biological range,  
240 direct comparisons are not made for this metric. Future measurements, using a  
241 more sensitive HPLC method for quantitating serum Creatinine will improve our  
242 ability to detect more subtle changes.

243

244 In addition to characterizing baseline electrolytes and their response to  
245 experimental dehydration, the normative value for daily water intake was  
246 estimated to be 0.11 mL per day per gram body weight. Though comparable measures  
247 of water consumption are scarce, one study in two arid adapted *Limoys* (*L.*  
248 *pictus* and *L. irroratus*) housed in non-desert captive settings were estimated  
249 to be 0.18 and 0.17 mL per day per gram body weight respectively (17) - a value  
250 much greater than in *P. eremicus*.

251

252 Animals that were exposed to experimental dehydration lost a substantial amount  
253 of body weight. Dehydration in humans, resulting in loss of even a fraction of  
254 this amount results in cardiovascular collapse and death (40). Indeed, even a  
255 dehydration-related loss of a few percent of body weight may cause serious  
256 renal impairment or renal failure. That the cactus mouse may lose so much  
257 weight as a result of dehydration and remain active, and apparently healthy,  
258 without renal impairment is a testament to their desert adaptation. The  
259 magnitude of weight loss and the negative (though non-significant) relationship  
260 between baseline weight and weight loss, coupled with the lack of behavioral  
261 impairments suggests that metabolic water production via the oxidation of fat  
262 may be an important and potentially adaptive mechanism preventing more serious



263 complications from acute dehydration. Indeed, water metabolism may produce a  
264 substantial amount of water (reviewed in (31)), and had been demonstrated in a  
265 diverse group of animals including marine mammals (49) and desert rodents (20).  
266 Future studies of fat metabolism in *P. eremicus*, using computed tomography,  
267 are planned. Because animals are essentially anuric, particularly when  
268 dehydrated, means direct measurement of fax oxidation (e.g., urine beta  
269 hydroxybutryate) is not possible.

270

271 As described above, mice appear grossly behaviorally intact. Despite this, they  
272 may be experiencing a degree of cognitive impairment, as is the case with human  
273 dehydration, where even mild-dehydration is associated with cognitive  
274 impairment (4). Future studies, using classical Y-maze and novel object  
275 recognition tests aim to understand more fully the cognitive effects of  
276 dehydration in cactus mouse.

277

278 In addition to weight loss, dehydrated animals demonstrated biochemical  
279 evidence of physiological stress, in the form of increased Sodium, Chloride,  
280 BUN, and Bicarb. There were no significant relationships between any  
281 physiological value and Creatinine, suggesting that dehydration related stress  
282 does not result in renal impairment or damage. Indeed, this is in contrast to  
283 humans and other mammals where acute dehydration of the nature imposed on these  
284 animals is universally related to renal failure and subsequent death. That *P.*  
285 *eremicus* can withstand this level of dehydration is a testament to the processes  
286 involved in adaptation.

287

288 In summary, we present here a set of physiological measurements that represent  
289 the endpoints in the physiological management of acute dehydration in the  
290 desert adapted cactus mouse. How these endpoint are achieved is an outstanding  
291 question deserving future study, particularly in light of global climate change  
292 (31). For instance Vasopressin, along with the Renin-Angiotensin-Aldosterone  
293 system are thought to be a critically important to the regulation of water and  
294 solute balance (6, 45, 47, 60, 63). Comparative genomic analysis, studies of

295 gene expression, and the measurement of protein levels will provide important  
296 insights into the actual mechanisms underlying these phenotypes.

297

298 In addition to understanding the mechanisms of salt and water balance,  
299 characterizing the ways in which desert animals prevent dehydration-linked  
300 renal failure is exceptionally important. Unlike humans, where repeated  
301 dehydration events leads to a progressive decline in renal function (21), it  
302 is hypothesized that repeated acute-dehydration is unlikely to be linked to  
303 renal failure in animals that have evolved in desert environments. Testing this  
304 hypothesis, along with understanding the mechanisms which limit renal damage  
305 (e.g., modulating renal microcirculation, maintaining cell volume via organic  
306 osmolytes) could provide previously uncharacterized clinically-relevant  
307 insights into renal (dis)function.

308

309

- 310 1. **Adrogué HJ, Madias NE.** Hyponatremia. *New Engl J Med* 342: 1493–1499,  
311 2000.
- 312 2. **al-Kahtani MA, Zuleta C, Caviedes-Vidal E, Garland T.** Kidney mass and  
313 relative medullary thickness of rodents in relation to habitat, body  
314 size, and phylogeny. *Physiol Biochem Zool* 77: 346–365, 2004.
- 315 3. **Altschuler EM, Nagle RB, Braun EJ, Lindstedt SL, Krutzsch PH.**  
316 Morphological study of the desert heteromyid kidney with emphasis on the  
317 genus *Perognathus*. *Anat Rec* 194: 461–468, 1979.
- 318 4. **Armstrong LE, Ganio MS, Casa DJ, Lee EC, McDermott BP, Klau JF, Jimenez  
319 L, Le Bellego L, Chevillotte E, Lieberman HR.** Mild dehydration affects  
320 mood in healthy young women. *Journal of Nutrition* 142: 382–388, 2012.
- 321 5. **Aroch I, King R, Baneth G.** Hematology and serum biochemistry values of  
322 trapped, healthy, free-ranging rock hyraxes (*Procavia capensis*) and  
323 their association with age, sex, and gestational status. *Vet Clin Pathol*  
324 36: 40–48, 2007.
- 325 6. **Bankir L, Fernandes S, Bardoux P, Bouby N, Bichet DG.** Vasopressin-V2  
326 receptor stimulation reduces sodium excretion in healthy humans. *J Am  
327 Soc Nephrol* 16: 1920–1928, 2005.
- 328 7. **Barrett JM, Kriz W, Kaissling B, De Rouffignac C.** The ultrastructure of  
329 the nephrons of the desert rodent (*Psammomys obesus*) kidney. II. Thin  
330 limbs of Henle of long-looped nephrons. *Am J Anat* 151: 499–514, 1978.

- 331 8. **Baum N, Dichoso CC, Carlton CE.** Blood urea nitrogen and serum  
332 creatinine. *Physiology and interpretations. Urology* 5: 583–588, 1975.
- 333 9. **Bedford NL, Hoekstra HE.** Peromyscus mice as a model for studying natural  
334 variation. *eLife* 4:e06813, 2015.
- 335 10. **Beuchat CA.** Structure and concentrating ability of the mammalian kidney:  
336 correlations with habitat. *Am J Physiol* 271: R157–79, 1996.
- 337 11. **Blaustein MP, Lederer WJ.** Sodium/calcium exchange: its physiological  
338 implications. *Physiol Rev* 79: 763–854, 1999.
- 339 12. **Bradford DF.** Water stress of free-living *Peromyscus truei*. *Ecology* 55:  
340 1407–1414, 1974.
- 341 13. **Bradley RD, Durish ND, Rogers DS, Miller JR, Engstrom MD, Kilpatrick CW.**  
342 Toward a molecular phylogeny for *Peromyscus*: Evidence from mitochondrial  
343 cytochrome-b sequences. *J Mammal* 88: 1146–1159, 2007.
- 344 14. **Calisi RM, Bentley GE.** Lab and field experiments: Are they the same  
345 animal? *Horm Behav* 56: 1–10, 2009.
- 346 15. **Castoe TA, de Koning APJ, Hall KT, Card DC, Schield DR, Fujita MK,**  
347 **Ruggiero RP, Degner JF, Daza JM, Gu W, Reyes-Velasco J, Shaney KJ,**  
348 **Castoe JM, Fox SE, Poole AW, Polanco D, Dobry J, Vandewege MW, Li Q,**  
349 **Schott RK, Kapusta A, Minx P, Feschotte C, Uetz P, Ray DA, Hoffmann FG,**  
350 **Bogden R, Smith EN, Chang BSW, Vonk FJ, Casewell NR, Henkel CV,**  
351 **Richardson MK, Mackessy SP, Bronikowski AM, Yandell M, Warren WC, Secor**  
352 **SM, Pollock DD.** The Burmese python genome reveals the molecular basis  
353 for extreme adaptation in snakes. *PNAS* 110: 20645–20650, 2013.
- 354 16. **Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN.** Mechanisms of aerobic  
355 performance impairment with heat stress and dehydration. *Journal of*  
356 *Applied Physiology* 109: 1989–1995, 2010.
- 357 17. **Christian DP, Matson JO, Rosenberg SG.** Comparative water balance in two  
358 species of *Liomys*. *Comp Biochem Physiol, Part A Mol Integr Physiol* 61A:  
359 589–559, 1978.
- 360 18. **Costill DL, Coté R, Fink W.** Muscle water and electrolytes following  
361 varied levels of dehydration in man. *Journal of Applied Physiology* 40:  
362 6–11, 1976.
- 363 19. **Feig PU, McCurdy DK.** The hypertonic state. *N Engl J Med* 297: 1444–1454,  
364 1977.
- 365 20. **Frank CL.** Diet Selection by a Heteromyid Rodent: Role of Net Metabolic  
366 Water Production. *Ecology* 69: 1943–1951, 1988.
- 367 21. **Glaser J, Lemery J, Rajagopalan B, Diaz HF, García-Trabanino R, Taduri**  
368 **G, Madero M, Amarasinghe M, Abraham G, Anutrakulchai S, Jha V,**

- 369        **Stenvinkel P, Roncal-Jimenez C, Lanaspá MA, Correa-Rotter R, Sheikh-**  
370        **Hamad D, Burdmann EA, Andres-Hernando A, Milagres T, Weiss I, Kanbay M,**  
371        **Wesseling C, Sánchez-Lozada LG, Johnson RJ.** Climate Change and the  
372        Emergent Epidemic of CKD from Heat Stress in Rural Communities: The Case  
373        for Heat Stress Nephropathy. *Clinical Journal of the American Society of*  
374        *Nephrology* 11: 1472–1483, 2016.
- 375    22.   **Haussinger D.** The role of cellular hydration in the regulation of cell  
376        function. *Biochem J* 313 ( Pt 3): 697–710, 1996.
- 377    23.   **Hayes J, Bible C, Boone J.** Repeatability of mammalian physiology:  
378        Evaporative water loss and oxygen consumption of *Dipodomys merriami*. *J*  
379        *Mammal* 79: 475–485, 1998.
- 380    24.   **Heimeier RA, Davis BJ, Donald JA.** The effect of water deprivation on the  
381        expression of atrial natriuretic peptide and its receptors in the  
382        spinifex hopping mouse, *Notomys alexis*. *Comparative Biochemistry and*  
383        *Physiology Part A: Physiology* 132: 893–903, 2002.
- 384    25.   **Hoekstra H, Moghadam HK, Hoekstra J, Harrison PW, Berrigan D, Zachar G,**  
385        **Székely T, Vignieri S, Mank JE, Hoang A, Hill C, Beerli P, Kingsolver J.**  
386        Strength and tempo of directional selection in the wild. *P Natl Acad Sci*  
387        *Usa* 98: 9157–9160, 2001.
- 388    26.   **Howell AB, Gersh I.** Conservation of water by the rodent *Dipodomys*. *J*  
389        *Mammal* 16: 1, 1935.
- 390    27.   **Huang Y, Tracy R, Walsberg GE, Makkinje A, Fang P, Brown D, Van Hoek AN.**  
391        Absence of aquaporin-4 water channels from kidneys of the desert rodent  
392        *Dipodomys merriami merriami*. *Am J Physiol-Renal* 280: F794–F802, 2001.
- 393    28.   **Huerta-Sánchez E, Jin X, Asan, Bianba Z, Peter BM, Vinckenbosch N, Liang**  
394        **Y, Yi X, He M, Somel M, Ni P, Wang B, Ou X, Huasang, Luosang J, Cuo ZXP,**  
395        **Li K, Gao G, Yin Y, Wang W, Zhang X, Xu X, Yang H, Li Y, Wang J, Wang J,**  
396        **Nielsen R.** Altitude adaptation in Tibetans caused by introgression of  
397        Denisovan-like DNA. *Nature* 512: 194–197, 2014.
- 398    29.   **Jentsch TJ, Stein V, Weinreich F, Zdebik AA.** Molecular Structure and  
399        Physiological Function of Chloride Channels. *Physiol Rev* 82: 503–568,  
400        2002.
- 401    30.   **Jéquier E, Constant F.** Water as an essential nutrient: the physiological  
402        basis of hydration. *Eur J Clin Nutr* 64: 115–123, 2009.
- 403    31.   **Johnson RJ, Johnson RJ, Stenvinkel P, Stenvinkel P, Jensen T, Jensen T,**  
404        **Lanaspá MA, Lanaspá MA, Roncal C, Roncal C, Song Z, Song Z, Bankir L,**  
405        **Bankir L, Sánchez-Lozada LG, Sanchez-Lozada LG.** Metabolic and Kidney  
406        Diseases in the Setting of Climate Change, Water Shortage, and Survival  
407        Factors. *Journal of the American Society of Nephrology* 27: 2247–2256,  
408        2016.

- 409 32. **MacManes MD, Eisen MB.** Characterization of the transcriptome, nucleotide  
410 sequence polymorphism, and natural selection in the desert adapted mouse  
411 *Peromyscus eremicus*. *PeerJ* 2: e642, 2014.
- 412 33. **MacManes MD, Lacey EA.** Is promiscuity associated with enhanced selection  
413 on MHC-DQ $\alpha$  in mice (genus *Peromyscus*)? *PLOS ONE* 7: e37562, 2012.
- 414 34. **MacMillen RE, Lee AK.** Australian desert mice: independence of exogenous  
415 water. *Science* 158: 383–385, 1967.
- 416 35. **Mares M.** Water Economy and Salt Balance in a South-American Desert  
417 Rodent, *Eligmodontia typus*. *Comp Biochem Phys A* 56: 325–332, 1977.
- 418 36. **Marra NJ, Eo SH, Hale MC, Waser PM, DeWoody A.** A priori and a posteriori  
419 approaches for finding genes of evolutionary interest in non-model  
420 species: Osmoregulatory genes in the kidney transcriptome of the desert  
421 rodent *Dipodomys spectabilis* (Banner-Tailed Kangaroo Rat). *Comparative*  
422 *Biochemistry and Physiology - Part D: Genomics and Proteomics* (July 26,  
423 2012). doi: 10.1016/j.cbd.2012.07.001.
- 424 37. **Marra NJ, Romero A, DeWoody JA.** Natural selection and the genetic basis  
425 of osmoregulation in heteromyid rodents as revealed by RNA-seq. *Mol Ecol*  
426 23: 2699–2711, 2014.
- 427 38. **Mbassa GK.** Mammalian renal modifications in dry environments. *Vet Res*  
428 *Commun* 12: 1–18, 1988.
- 429 39. **McKinney TD, Burg MB.** Bicarbonate and fluid absorption by renal proximal  
430 straight tubules. *Kidney Int* 12: 1–8, 1977.
- 431 40. **Mehta RL, Cerdá J, Burdmann EA, Tonelli M, García-García G, Jha V,**  
432 **Susantitaphong P, Rocco M, Vanholder R, Sever MS, Cruz D, Jaber B,**  
433 **Lameire NH, Lombardi R, Lewington A, Feehally J, Finkelstein F, Levin N,**  
434 **Pannu N, Thomas B, Aronoff-Spencer E, Remuzzi G.** International Society  
435 of Nephrology's 0by25 initiative for acute kidney injury (zero  
436 preventable deaths by 2025): a human rights case for nephrology. *Lancet*  
437 385: 2616–2643, 2015.
- 438 41. **Merkt JR, Taylor CR.** “Metabolic switch” for desert survival. *P Natl Acad*  
439 *Sci Usa* 91: 12313–12316, 1994.
- 440 42. **Montain S, Latzka W, Sawka N.** Fluid replacement recommendations for  
441 training in hot weather. *Mil Med* 164: 502–508, 1999.
- 442 43. **Moreau B, Vié JC, Cotellon P, Thoisy ID, Motard A, Raccurt CP.**  
443 HEMATOLOGIC AND SERUM BIOCHEMISTRY VALUES IN TWO SPECIES OF FREE-RANGING  
444 PORCUPINES (*COENDOU PREHENSILIS*, *COENDOU MELANURUS*) IN FRENCH GUIANA. *J*  
445 *Zoo Wildl Med* 34: 159–162, 2003.
- 446 44. **Mullen LM, Vignieri SN, Gore JA, Hoekstra HE.** Adaptive basis of  
447 geographic variation: genetic, phenotypic and environmental differences

- 448 among beach mouse populations. *Proc Biol Sci* 276: 3809–3818, 2009.
- 449 45. **Muñoz A, Riber C, Trigo P.** Dehydration, electrolyte imbalances and  
450 renin-angiotensin-aldosterone-vasopressin axis in successful and  
451 unsuccessful endurance horses *Equine Veterinary Journal - Wiley Online*  
452 *Library. Equine Veterinary ...* (2010). doi: 10.1111/j.2042-  
453 3306.2010.00211.x/pdf.
- 454 46. **Nagy KA.** Seasonal patterns of water and energy balance in desert  
455 vertebrates. *J Arid Environ* 14: 201–210, 1988.
- 456 47. **Nielsen S, Chou C, Marples D, Christensen E, Kishore B, Knepper M.**  
457 Vasopressin increases water permeability of kidney collecting duct by  
458 inducing translocation of aquaporin-CD water channels to plasma-  
459 membrane. *P Natl Acad Sci Usa* 92: 1013–1017, 1995.
- 460 48. **Nssien M, Olayemi FO, Onwuka SK, Olusola A.** Comparison of some plasma  
461 biochemical parameters in two generations of african giant rat  
462 (*Cricetomys gambianus*, waterhouse). *African Journal of Biomedical*  
463 *Research* 5, 2002.
- 464 49. **Ortiz RM.** Osmoregulation in marine mammals. *Journal of Experimental*  
465 *Biology* 204: 1831–1844, 2001.
- 466 50. **R Core Development Team F.** R: A Language and Environment for Statistical  
467 Computing [Online]. R Development Core Team: 2011. [http://www.R-](http://www.R-project.org)  
468 [project.org](http://www.R-project.org).
- 469 51. **Schmidt-Nielsen B, Schmidt-Nielsen K.** Pulmonary Water Loss in Desert  
470 Rodents. *Am J Physiol* 162: 31–36, 1950.
- 471 52. **Shorter KR, Crossland JP, Webb D, Szalai G, Felder MR, Vrana PB.**  
472 *Peromyscus* as a Mammalian Epigenetic Model. *Genetics Research*  
473 *International* 2012: 1–11, 2012.
- 474 53. **Sikes RS, Gannon WL, Animal Care and Use Committee of the American**  
475 **Society of Mammalogists.** Guidelines of the American Society of  
476 Mammalogists for the use of wild mammals in research. *J Mammal* 92: 235–  
477 253, 2011.
- 478 54. **Steiner CC, Weber JN, Hoekstra HE.** Adaptive variation in beach mice  
479 produced by two interacting pigmentation genes. *PLoS Biol* 5: e219, 2007.
- 480 55. **Thornton PC, Wright PA, Sacra PJ, Goodier TE.** The ferret, *Mustela*  
481 *putorius furo*, as a new species in toxicology. *Lab Anim* 13: 119–124,  
482 1979.
- 483 56. **Tracy R, Walsberg G.** Intraspecific variation in water loss in a desert  
484 rodent, *Dipodomys merriami*. *Ecology* 82: 1130–1137, 2001.
- 485 57. **Veal R, Caire W.** *Peromyscus eremicus*. *Mammalian Species* 118: 1–6, 2001.

- 486 58. **Viggers KL, Lindenmayer DB.** Variation in Hematological and Serum  
487 Biochemical Values of the Mountain Brushtail Possum, *Trichosurus caninus*  
488 Ogilby (Marsupialia: Phalangeridae). *J Wildlife Dis* 32: 142-146, 1996.
- 489 59. **Vignieri SN, Larson JG, Hoekstra HE.** The selective advantage of crypsis  
490 in mice. *Evolution* 64: 2153-2158, 2010.
- 491 60. **Weaver D, Walker L, Alcorn D, Skinner S.** The contributions of renin and  
492 vasopressin to the adaptation of the Australian spinifex hopping mouse  
493 (*Notomys alexis*) to free water deprivation. *Comp Biochem Physio* 108:  
494 107-116, 1994.
- 495 61. **Weber DK, Danielson K, Wright S, Foley JE.** Hematology and serum  
496 biochemistry values of dusky-footed wood rat (/). *J Wildlife Dis* 38:  
497 576-582, 2002.
- 498 62. **Yu FH, Catterall WA.** Overview of the voltage-gated sodium channel  
499 family. *Genome Biology* 4: 207, 2003.
- 500 63. **Zuber AM, Singer D, Penninger JM, Rossier BC, Firsov D.** Increased renal  
501 responsiveness to vasopressin and enhanced V2 receptor signaling in  
502 RGS2-/- mice. *J Am Soc Nephrol* 18: 1672-1678, 2007.

503 Table 1

504

	Normal	Min	Max	Mean
Sodium (mMol/L)	148-158	144	170	153
Chloride (mMol/L)	110-115	105	126	113
BUN (mg/dL)	29-46	22	64	37
Bicarb (mMol/L)	19-25	15	26	22
Creatinine (mg/dL)	>0.2-0.3	>0.2	0.4	0.22

505

506 **Table 1.** Normal values for serum electrolytes. Normal values (n=44, 24 male,  
507 20 female) are defined as those values falling between the 1<sup>st</sup> and 3<sup>rd</sup> quartile.  
508 Of note, the Abaxis VS2 electrolyte analyzer does not measure Creatinine below

509 0.2 mg/dL; therefore, the range for normal Creatinine is truncated at this  
510 value.

511

512

513

514

515

516

517

518

519

520

521

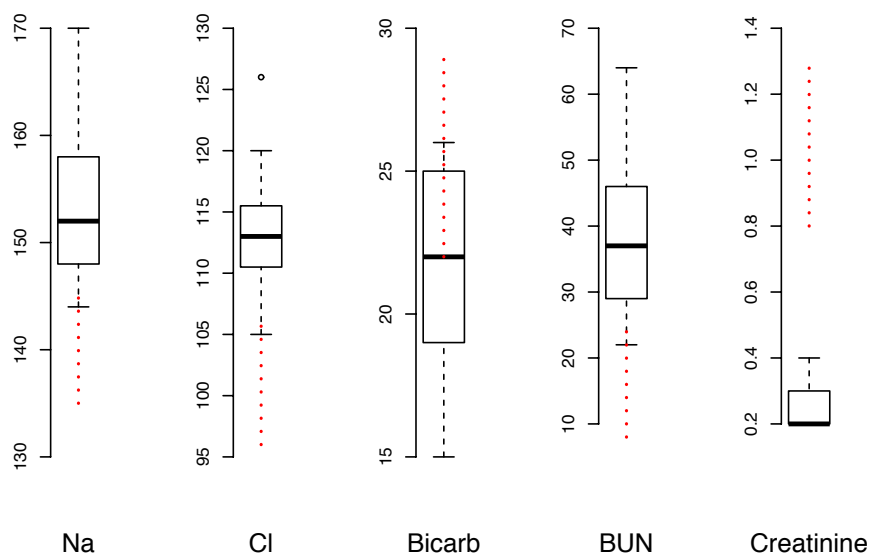
522

523

524 **Figure 1.** Normal values (n=44, 24 male, 20 female) for serum electrolytes.  
525 Human normal values (from Medline) are plotted for comparison in dotted red  
526 lines. Of note, the Abaxis VS2 electrolyte analyzer does not measure Creatinine  
527 below 0.2 mg/dL, and therefore the range for normal Creatinine is truncated at  
528 this value.

529





530

531

532

533

534

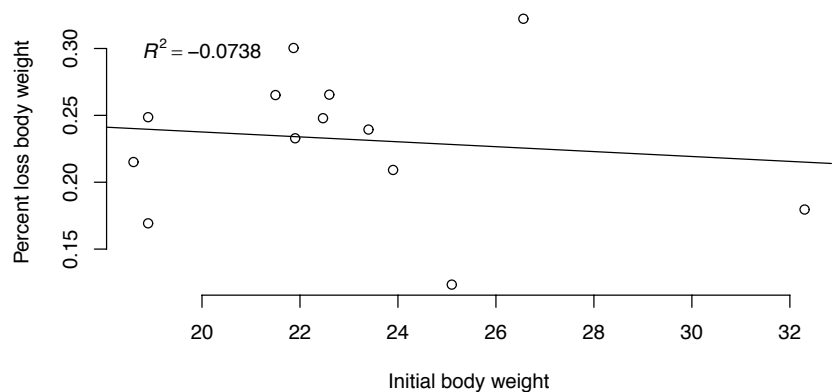
535

536

537

538 **Figure 2.** Percent body weight loss as a function of initial body weight due to  
539 experimental dehydration. No significant trend exists. (n=13, 7 males, 6  
540 females)

541



542

543

544

545

546

547

548

549

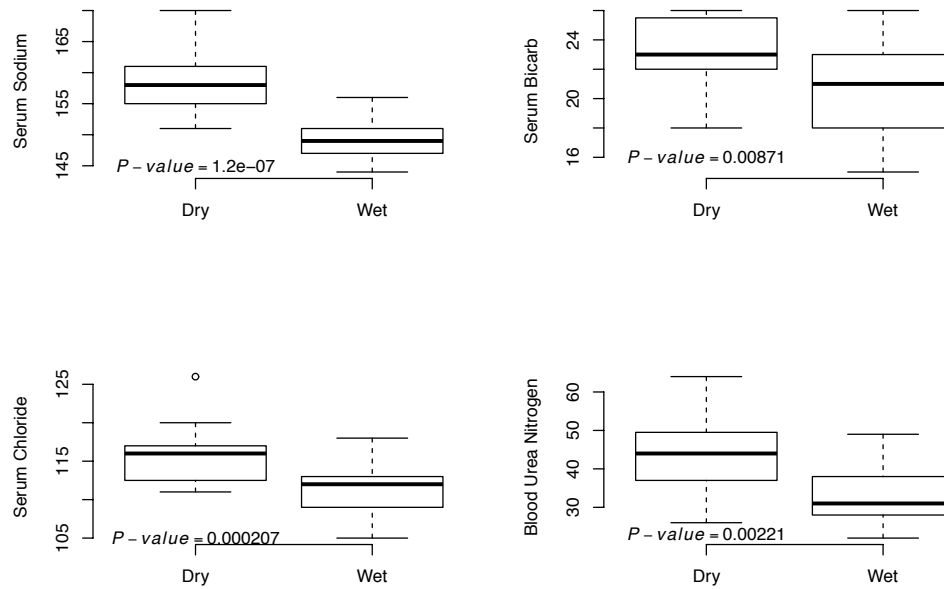
550

551

552

553 **Figure 3.** Experimental dehydration resulted in increases in serum sodium,  
554 chloride, BUN and bicarbonate ion. Reported p-values are from a two-tailed t-  
555 test (n=13 dehydrated: DRY, n=31 hydrated: Wet)

556



557

558

559

560

561

562

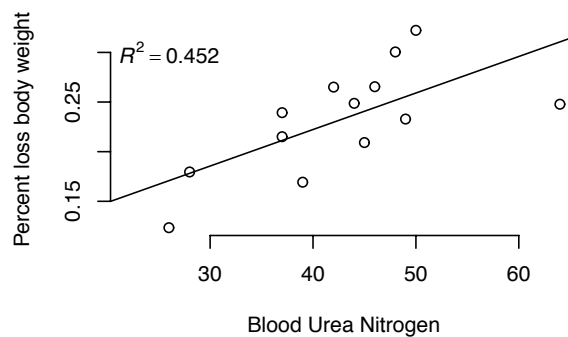
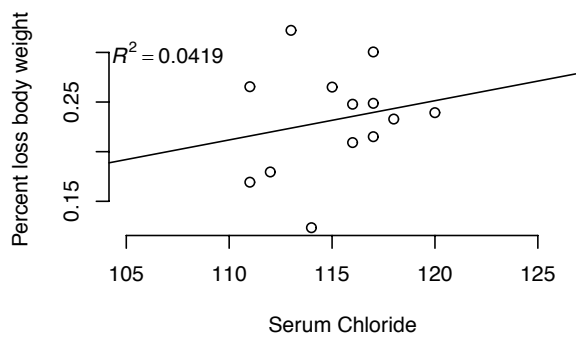
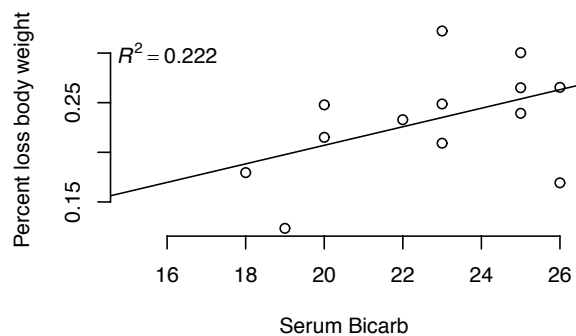
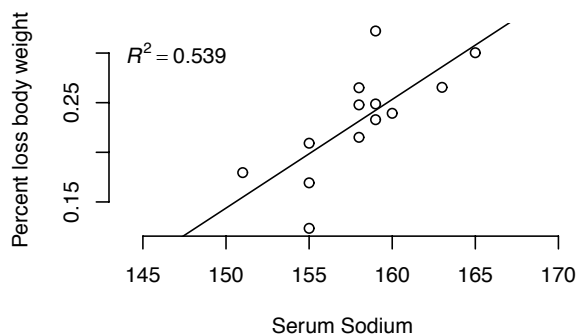
563

564

565

566

567 **Figure 4.** The relationship between serum electrolytes is positive in all cases  
568 and significant for Sodium (F-statistic: 12.85, 11 DF, p-value: 0.004283) and  
569 BUN (F-statistic: 9.089, 11 DF, p-value: 0.01177). n=13, 7 males, 6 females



570

571