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

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Abstract

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

Introduction

Understanding the evolution of adaptive traits has long been one of the primary goals in evolutionary biology. The study of the relationships between fitness and phenotype, often powered by modern genomic techniques (Vignieri et al. 2010), has provided researchers with insight into the mechanistic processes that underlie adaptive phenotypes (Castoe et al. 2013; Huerta-Sánchez et al. 2014). Systems in which the power of genomics can be combined with an understanding of natural history and physiology are well suited for the study of adaptation (Mullen et al. 2009; Bedford and Hoekstra 2015) especially when researchers have the ability to assay the link between genotype and phenotype in wild animals and then conduct complementary experiments using representative animals in carefully controlled laboratory environments. The study described here, characterizing the physiology and serum biochemistry of *Peromyscus eremicus* is the first step in a larger study aimed at understanding the genomics architecture of adaptation to desert environments.

Desert adaptation has significant ecological, evolutionary, and biomedical significance. In contrast to humans and other mammals, desert rodents can survive in extreme environmental conditions and are resistant to the effects of dehydration. Physiological adaptions to deserts have been characterized in several rodents. Specifically, renal histology has been studied in multiple Heteromyid rodents (Altschuler et al. 1979), and the general conclusion is that these desert adapted animals have evolved elongate Loops of Henle (Barrett et al. 1978; Mbassa 1988; Beuchat 1996) that are hypothesized to optimize water conservation. In addition to studies of renal histology, several studies have characterized pulmonary water loss (Schmidt-Nielsen and Schmidt-Nielsen 1950; Hayes et al. 1998), water metabolism (Howell and Gersh 1935), and water consumption (MacMillen and Lee 1967; Bradford 1974; Mares 1977; Nagy 1988; Merkt and Taylor 1994) in desert rodents. While desert animals possess specialized physiology that is efficient with regards to water metabolism and loss, whether or not specialized

genomic adaptation exists is an active area of research (Marra et al. 2012; MacManes and Eisen 2014; Marra et al. 2014) .

Although the cactus mouse (*Peromyscus eremicus*) has not been a particular focus for the study of desert adaptation (but see (al-Kahtani et al. 2004; MacManes and Eisen 2014), this Cricetid rodent native to the arid regions of the Southwestern United States and Northern Mexico (Veal and Caire 2001) offers a unique opportunity to understand physiological adaptations to deserts. *P. eremicus* is a member of a larger genus of animals known colloquially as the "*Drosophila* of mammals" (Bedford and Hoekstra 2015), and *Peromyscus* species have been the focus of extensive study (Hoekstra et al. 2001; Steiner et al. 2007; MacManes and Lacey 2012; Shorter et al. 2012). *P. eremicus* is a sister species to the non-desert adapted *P. californicus* (Bradley et al. 2007), and it is closely related to *P. crinitus*, the canyon mouse, which is another desert adapted rodent native to Southwestern deserts.

Critical to desert survival is the ability to maintain water balance even when the loss of water exceeds dietary water intake (Heimeier et al. 2002). Indeed, the mammalian corpus consists of 60% water (Jéquier and Constant 2009). Far from a static reservoir, proper physiologic function requires water for numerous processes, including nutrient transport (Haussinger 1996), signal transduction, pH balance, thermal regulation (Montain et al. 1999) and the removal of metabolic waste. To accomplish these functions, a nearly constant supply of water is required to replace water loss (Jéquier and Constant 2009), which occurs mainly via the gastrointestinal and genitourinary systems, and evaporative loss, which is greatly accelerated in extreme heat and aridity (Cheuvront et al. 2010). Because the body possesses limited reserves, when loss exceeds intake during even a short period of time, dehydration and death can occur. Mammals are exquisitely sensitive to dehydration and possess limited compensatory mechanisms.

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75 Characterizing desert adaptation requires careful and integrative physiological studies, which should
76 include a detailed characterization of water intake, responses to dehydration, and the measurement of
77 blood electrolytes. Indeed, quantifying these metrics is one of the first steps in understanding how
78 animals survive in the extreme heat and aridity of deserts. In particular, the electrolytes chloride and
79 sodium are important markers of dehydration (Costill et al. 1976). These molecules play essential roles
80 in metabolic and physiological processes, and they are integral to the functionally of a variety of
81 transmembrane transport pumps (Blaustein and Lederer 1999; Jentsch et al. 2002), neurotransmission
82 (Yu and Catterall 2003), and maintenance of tonicity (Feig and McCurdy 1977). Furthermore,
83 hypernatremia causes restlessness, lethargy, muscle weakness, or coma (Adrogué and Madias 2000).
84 Bicarbonate ion, in contrast, is primarily responsible for aiding in the maintenance of the acid-base
85 balance and is resorbed in the renal tubules (McKinney and Burg 1977). Blood urea nitrogen (BUN) is a
86 test that assays the abundance of urea – the end product for metabolism of nitrogen containing
87 compounds. Urea is resorbed in the glomerulus, and renal impairment is often inferred when BUN
88 becomes elevated (Baum et al. 1975). Importantly, the canonical model of urea resorption is dependent
89 on urine volume, which is markedly diminished in desert rodents, thus limiting the utility of using BUN
90 as an indicator of renal function. Lastly, creatinine, a product of muscle breakdown, whose measured
91 level does not depend on urine volume is used as a measure of renal function (Baum et al. 1975).

92

93 Genes most frequently implicated in desert-adaptation include members of the aquaporin family (Huang
94 et al. 2001). However, previous work suggests that an alternative gene family, the solute carriers, are
95 more relevant for desert-adaptation in the cactus mouse (MacManes and Eisen 2014). As a first step
96 towards fully elucidating the patterns of adaptive evolution to deserts in *P. eremicus*, we characterized
97 the normal patterns of water intake and electrolyte levels as well as the physiologic response to

98 experimental dehydration. As such, this study provides critical physiological and biochemical
99 information about *P. eremicus* and its response to dehydration and is generally useful as researchers
100 begin to leverage large-scale genome data against classic questions regarding the evolution of adaptive
101 phenotypes.
102
103 **Materials and Methods**
104 We used captive *P. eremicus* that were purchased from the University of South Carolina Peromyscus
105 Genetic Stock Center in 2013. These animals, which are descendant from wild caught animals from a
106 dry-desert population in Arizona, have been bred in captivity at the University of New Hampshire.
107 Animals are housed in a large walk-in environmental chamber built to replicate the environmental
108 conditions in which this population has evolved. Specifically, the animals experience a normal diurnal
109 pattern of temperature fluctuation, ranging from 90F during the daytime to 75F during the night.
110 Relative humidity (RH) ranges from 10% during the day to 25% during the night. Animals are housed in
111 standard lab mouse cages with bedding that has been dehydrated to match desert conditions. They are
112 fed a standard rodent chow, which has also been dehydrated. Water is provided *ad lib* during certain
113 phases of experimentation and withheld completely during others. All animal care procedures follow
114 the guidelines established by the American Society of Mammalogy (Sikes et al. 2011) and have been
115 approved by the University of New Hampshire Animal Care and Use Committee under protocol number
116 103092.
117
118 All animals included in this study were sexually mature adults. A slight bias for the inclusion of males
119 exists, as a concurrent study of male reproductive genomics was occurring. Preliminary analyses
120 conducted suggest that no significant differences in any of the physiological measures, and as a result,
121 males and females were analyzed as one group. For a subset of animals, water intake was measured,

which was accomplished via the use of customized 15ml conical tubes, wherein water intake was measured every 24 hours for a minimum of 3 consecutive days (range 3-10 days). Animals selected for the dehydration trial were weighed on a digital scale, housed without water for three days, then re-weighed to determine the change in body mass due to dehydration. At the conclusion of water measurement or after a three-day dehydration animals were sacrificed via isoflurane overdose and decapitation. Immediately after death, a 120uL sample of trunk blood was obtained for serum electrolyte measurement. This was accomplished using an Abaxis Vetscan VS2 machine with a critical care cartridge, which measures the concentration of several electrolytes (Sodium, Chloride, Bicarbonate ion, Creatinine, and Blood Urea Nitrogen (BUN)) relevant to hydration status and renal function. Lastly, the kidney, spleen, liver, lung, hypothalamus, testes, vas deferens and epididymis were dissected out and stored in RNAlater (Ambion Inc.) for future study. All statistical analyses were carried out in the statistical package, R (R Core Development Team 2011).

Results

We measured the daily water intake for 22 adult cactus mice for between three and 10 consecutive days. Mean water intake was 0.11 mL per day per gram body weight (median=0.11, SD= 0.05, min= 0.033, max=0.23). We measured levels of serum Sodium, Chloride, Bicarbonate ion, Creatinine, and Blood Urea Nitrogen (BUN) for 44 adult mice, thereby establishing normal (baseline) values for *P. eremicus* (Figure 1 and Table 1).

A comparison of mice provided with water *ad libitum* to mice that exposed to experimental water deprivation for three days revealed that the dehydrated mice lost an average of 23.2% of their body weight (median=23.9%, SD=5.3%, min=12.3%, max=32.3%). Despite this substantial weight loss, anecdotally, mice appeared healthy. They were active, eating, and interacting normally. The amount of

146 weight loss did not depend on daily water intake (p=0.63, R^2= 0.03), though the trend suggests that
147 animals that drink more water lost more weight). Furthermore, body weight did not strongly influence
148 the percent loss of body weight (Figure 2; p=0.68, R^2= 0.02).
149
150 In addition to a substantial loss in body weight, dehydration was associated with differences in serum
151 electrolytes (Figure 3; n=19 dehydrated, n=24 hydrated). These changes were subtle, but significant
152 using a two-sample t-test ($p < 0.008$ in all cases).
153
154 Lastly, the levels of serum electrolytes were tightly correlated with percent body weight loss (Figure 4).
155 Indeed, the relationship between the level of serum sodium and weight loss was positive and significant,
156 (ANOVA, F-statistic: 12.85, 11 DF, p= 0.004), as was the relationship between BUN and weight loss
157 (ANOVA, F-statistic: 9.089, 11 DF, p= 0.012). The relationships between weight loss and chloride and
158 bicarbonate levels respectively, were positive but not significant.
159
160 **Discussion**
161 Deserts are amongst the harshest environments on the planet. Indeed, animals living in these areas
162 must be highly adapted to the unique combination of extreme heat and aridity. Given that our
163 understanding of the physiology of desert adapted animals is limited largely to studies in renal histology
164 (Mbassa 1988) and on water intake and output (MacMillen and Lee 1967; Tracy and Walsberg 2001), an
165 enhanced understanding of serum electrolyte changes due to dehydration is informative. Because many
166 of the harmful effects of dehydration result from electrolyte abnormalities, characterizing normal values
167 and the electrolyte response to dehydration represents a critical first step in garnering a deeper
168 understanding of how desert animals survive despite severe and prolonged dehydration.
169

In this study, normal (baseline) values for serum Sodium, Chloride, Bicarbonate Ion, Creatinine, and Blood Urea Nitrogen were established in a captive colony of lab animals housed in desert conditions. Although these measures may differ in wild animals (see (Calisi and Bentley 2009) for a brief review of such differences), establishing normal values in captive animals is crucial, though future studies aim to understand the patterns of electrolyte variation in wild animals. In *P. eremicus*, we define the normal ranges for each electrolyte as those values falling between the 1st and 3rd quartile. Serum Chloride and Sodium were significantly higher than in published ranges for other mammals, including humans, a marsupial (Viggers and Lindenmayer 1996), *Cricetomys* (Nssien et al. 2002), and the porcupine (Moreau et al. 2003). However, serum chloride and sodium levels in our study were quite comparable to another wild rodent, *Neotima fuscipes* (Weber et al. 2002), a Mustelid (Thornton et al. 1979), and the Hyrax (Aroch et al. 2007). Values for BUN are generally higher in this study; unfortunately, a direct comparison is not possible, as measured values are dependent on the volume of urine produced. Serum Creatinine is low, largely resulting from the general lack of muscle mass in *P. eremicus* relative to other mammals. However, because the equipment used to analyze this electrolyte does not effectively capture the lower end of the biological range, direct comparisons are not made for this metric.

In addition to characterizing baseline electrolytes and their response to experimental dehydration, the normative value for daily water intake was estimated to be 0.11 mL per day per gram body weight. Though comparable measures of water consumption are scarce, one study in two arid adapted *Limoys* (*L. pictus* and *L. irroratus*) housed in non-desert captive settings were estimated to be 0.18 and 0.17 mL per day per gram body weight respectively (Christian et al. 1978) – a value much greater than in *P. eremicus*.

Animals that were exposed to experimental dehydration lost a substantial amount of body weight. Dehydration in humans, resulting in loss of even a fraction of this amount results in cardiovascular collapse and death. Indeed, even a dehydration-related loss of a few percent of body weight may cause serious renal impairment or renal failure. That the cactus mouse may lose so much weight as a result of dehydration and remain active, and apparently healthy, without renal impairment is a testament to their desert adaptation. Yet, while anecdotally mice appear well, they may be experiencing substantial cognitive impairment, as is the case with mild-human dehydration (Armstrong et al. 2012). Future studies in the lab aim to understand the cognitive effects of dehydration in cactus mouse.

In addition to weight loss, dehydrated animals demonstrated biochemical evidence of physiological stress, in the form of increased Sodium, Chloride, BUN, and Bicarb. There were no significant relationships between any physiological value and Creatinine, suggesting that dehydration related stress does not result in renal impairment or damage. Indeed, this is in contrast to humans and other mammals where acute dehydration of the nature imposed on these animals is universally related to renal failure and subsequent death. That *P. eremicus* can withstand this level of dehydration is a testament to the processes involved in adaptation. Studies in progress aim to link patterns of physiological change of the types described here to patterns of gene expression in both captive and wild animals, further informing our understanding of renal failure due to dehydration in mammals.

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345 Table 1

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	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

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348 **Table 1**. Normal values for serum electrolytes. Normal values are defined as those values falling

349 between the 1st and 3rd quartile. Of note, the Abaxis VS2 electrolyte analyzer does not measure

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

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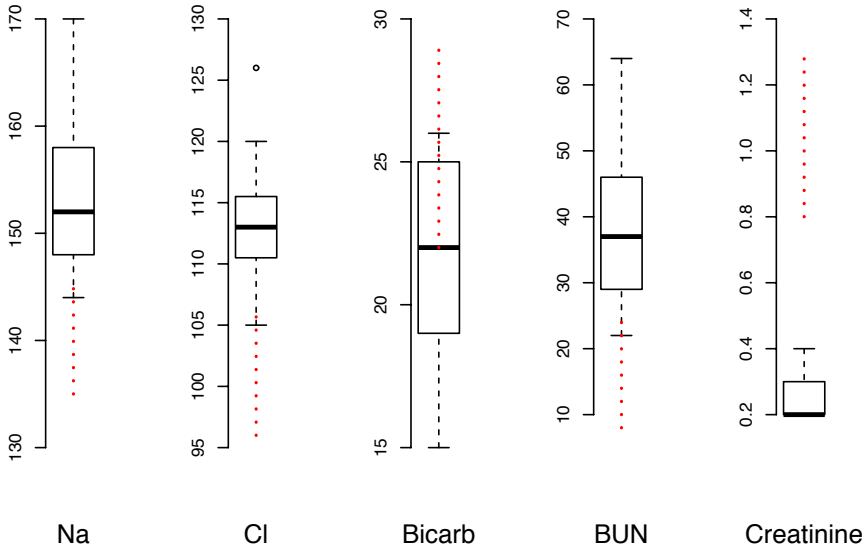
Figure 1. Normal values for serum electrolytes. Human normal values (from Medline) are plotted for comparison in dotted red lines. Of note, the Abaxis VS2 electrolyte analyzer does not measure Creatinine below 0.2 mg/dL, and therefore the range for normal Creatinine is truncated at this value.



369 **Figure 2**. Percent body weight loss as a function of initial body weight due to experimental dehydration.

370 No significant trend exists.

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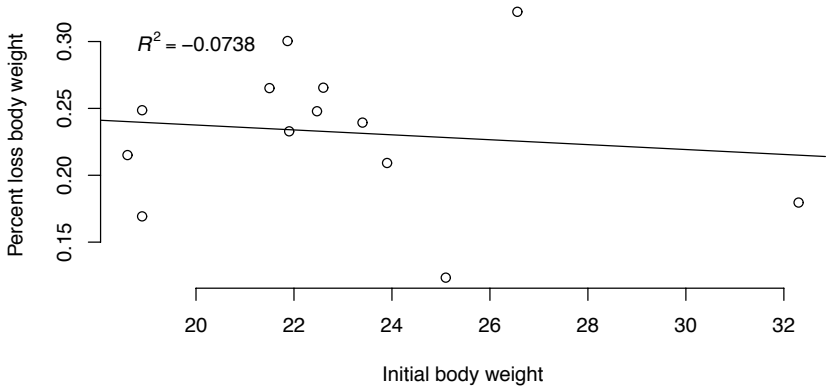

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382 **Figure 3**. Experimental dehydration resulted in increases in serum sodium, chloride, BUN and

383 bicarbonate ion. Reported p-values are from a two-tailed t-test (n=19 dehydrated: DRY, n=24 hydrated):

384 WET.

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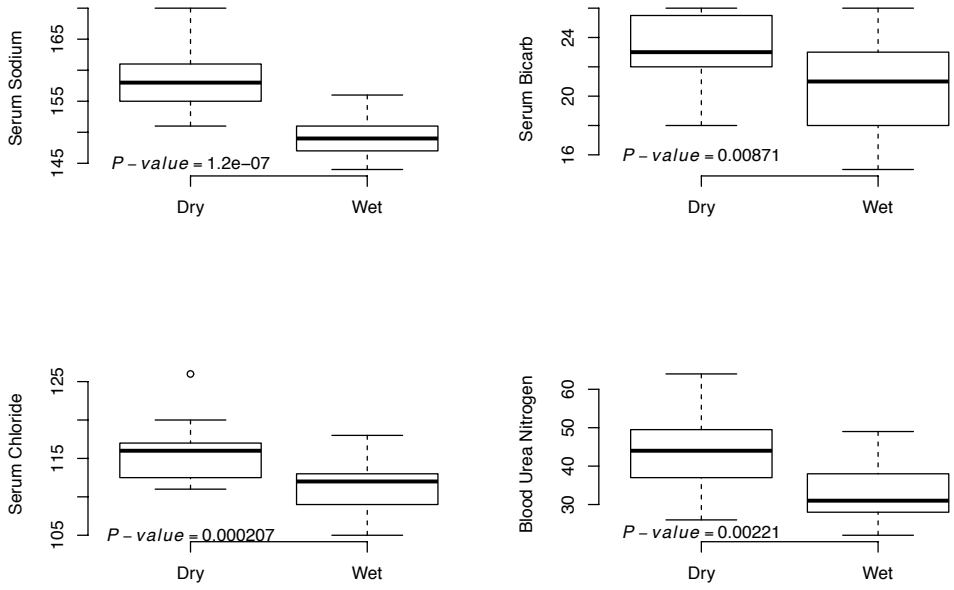

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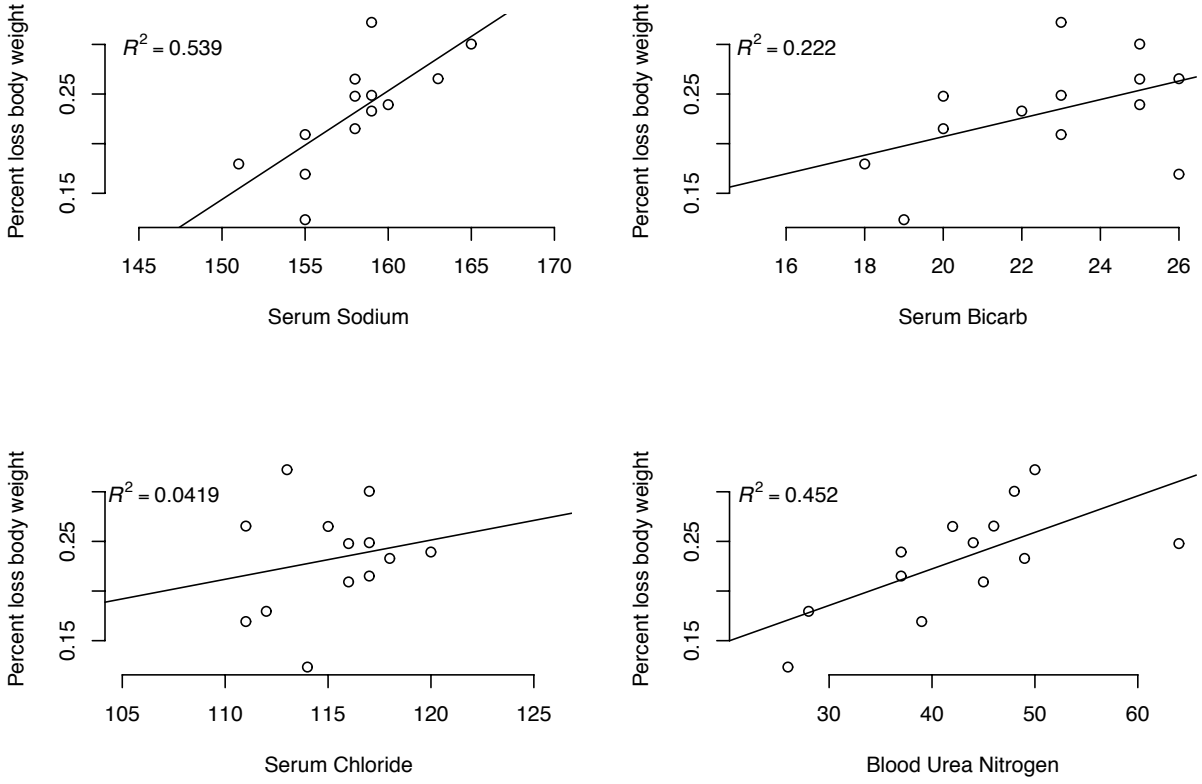
393 **Figure 4**. The relationship between serum electrolytes is positive in all cases and significant for Sodium

394 (F-statistic: 12.85, 11 DF, p-value: 0.004283) and BUN (F-statistic: 9.089, 11 DF, p-value: 0.01177)



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