High total water loss driven by low-fat diet in desert-adapted mice

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Abstract

Availability of essential food resources is one of the most important drivers of survival. Animals must either relocate or adapt to persist in environments where critical resource abundance is changing. Investigating a species' ability to respond to alternative conditions can reveal a myriad of responses. An optimal diet balances energy gain, water regulation, and nutrition. We used flow-through respirometry to characterize metabolic phenotypes of the desert-adapted cactus mouse (*Peromyscus eremicus*) under diurnally variable environmental conditions that mimic the environment of the Sonoran Desert. We treated mice with two different energy-controlled diets, a standard diet and a low-fat diet, and measured energy expenditure, rate of water loss, respiratory quotient, weight, and electrolyte levels. Mice fed the low-fat diet lost significantly more

water than those on the standard diet. Our results suggest that cactus mice may have limited capacity to tolerate water deprivation if optimal foods become less abundant. Given that climate change is predicted to modify the distribution of food items, understanding these links may have important implications for long-term population viability for desert and non-desert adapted animals alike.

Keywords: dietary fat, energy expenditure, *Peromyscus*, physiology, respirometry, total water loss

Introduction

As the planet undergoes unprecedented climate change, increased desertification is predicted (Wu et al. 2014; IPCC 2019; Mirzabaev et al. 2019). Desertification leads to increased aridity, a strong selective force that may result in changes to species distributions and community composition. Understanding these responses is critically important for predicting the impacts of climate change on biodiversity. Organismal responses to climate change vary, but can be classified into three general categories: geographic relocation to maintain physiological balance (Moritz et al. 2008; Walsh et al. 2016; Riddell et al. 2021), adaptation in place (particularly for species with fragmented habitats or limited dispersal ability, Reed et al. 2011), or perish, leading to population extirpation or extinction. Distributional changes in response to changing climate are well documented (Parmesan and Yohe 2003; Parmesan 2006; Moritz et al. 2008; Van der Putten et al. 2010; Pecl et al. 2017; Morrison et al. 2018). Adaptive responses can take two forms: evolutionary change over generational timeframes or phenotypic plasticity

within an animal's lifetime; however, the latter is less well documented. Adaptation *in situ* may involve morphological, behavioral, or physiological changes. For example, there are strong directional selections for seasonal adaptations (e.g., seasonal color camouflage, Jones et al. 2018), but in the absence of standing genetic variation, there is evidence of increased extinction risk (Spielman et al. 2004).

The Sonoran Desert is hot, dry, and environmentally dynamic. Daily ambient temperatures in the Summer can reach as high as 50°C during the day and as low as 15°C at night. In the winter, the daytime temperature is typically between 20 – 30°C and can fall to near freezing at night. Average relative humidity (RH) in the Sonoran Desert changes by 20% between seasons (Reid 1987; Unland et al. 1996; Sheppard et al. 2002, Boyd Deep Canyon Desert Research Center). High ambient temperatures and lack of extrinsic water challenge mammalian survival, as mammals rely on water for fundamental metabolic processes, behavior, and/or evaporative cooling (Reece et al. 2015). Despite these challenges, many mammals thrive in deserts. This has led to decades of work aimed at one central question: How do desert animals survive (Bradford 1974; MacMillen and Hinds 1983; Nagy 1987; Cortés et al. 2000; Kordonowy and MacManes 2017; MacManes 2017; Tigano et al. 2020; Bittner et al. 2021; Colella et al. 2021b)?

One of the most critical challenges faced by desert mammals includes the regulation of water balance. Water balance is maintained through a cascade of hormones that elicit a response designed to increase water retention and decreases water used in the excretion of nitrogenous waste. In brief, dehydration results in a decrease in blood volume and an increase in osmolality, the latter, an effect driven

largely by the rise in serum Sodium (Thornton 2010; Leib et al. 2016). The decrease in blood volume, detected by baroreceptors, signals the heart rate to increase in an attempt to maintain cardiac output and therefore, blood flow to critical organs (Kaufmann et al. 2020). In response to the increase in serum osmolality, osmoreceptors in the hypothalamus are stimulated and antidiuretic hormone (ADH) is released from the posterior pituitary gland (Thornton 2010; Leib et al. 2016). Then, several things happen; the kidneys are signaled to recover water (Fuller et al. 2020) and epithelial cells lining renal collecting tubules are signaled to move aquaporins from the interior of the cell to the surface. This increases the water permeability of cells and leads to more water reabsorbed from the tubule system back into the blood stream (Kortenoeven and Fenton 2014). The renin-angiotensin-aldosterone hormonal system in the kidneys is also signaled and spits into the active hormone angiotensin II, stimulating the release of aldosterone from the adrenal glands and ADH from the pituitary gland, resulting in the retention of sodium in the distal tubules of the nephrons (Goodfriend 2006). While hormonal regulation of water balance is paramount to survival, behavioral, physiological, and genomic responses are also critical.

Genomic and transcriptomic studies of desert mammals have found evidence of positive selection acting on genes involved in energy production, food storage (Jirimutu et al. 2012; Wu et al. 2014), water reabsorption (Jirimutu et al. 2012; Marra et al. 2012, 2014; Yang et al. 2016; Giorello et al. 2018), and osmoregulation (MacManes and Eisen 2014; Kordonowy and MacManes 2016; Colella et al. 2021b). A strong signature of positive selection in desert mice on Growth Differentiation Factor 10 (*Gdf10*) may prevent weight gain when consuming high-fat diets (Hino et al. 2017; Tigano et al.

2020). While this prior research has enhanced our understanding of the molecular processes underlying adaptation in desert environments, we still do not fully understand the suite strategies employed by desert animals to manage water efficiently.

Furthermore, animals that have evolved in desert environments are behaviorally and physiologically adapted to extreme aridity and lack of extrinsic water. Behaviors such as foraging strategies (Manlick et al. 2021), dietary preference (Frank 1988), burrowing (Schmidt-Nielsen and Adolph 1964; Louw and Seely 1984; Riddell et al. 2021), group nesting (Schmidt-Nielsen and Adolph 1964), microhabitat use during the heat of the day (Riddell et al. 2021), and nocturnality (but see Walsberg 2000; Tracy and Walsberg 2002) may optimize fitness in hot, dry environments by allowing animals to regulate temperature and water balance in real-time (Frank 1988). Behavioral changes may be the first line of defense against changing environments, as they are often dynamic, reversible, and happen in real time. In contrast, physiological adaptations, such as efficient kidney function or low fecal water content, which are observed in desert mammals to maintain water balance, can happen in real-time but typically have evolved over deeper evolutionary timescales (Christian and Matson 1978; Kordonowy and MacManes 2016).

Dietary intake of food is one important way in which animals may manage water. There are three main macromolecules that animals ingest as food items; carbohydrates, fats, and proteins, all of which vary in the amount of available energy and water, as well as in their cost to metabolize. Energy potential varies greatly, with carbohydrates and proteins yielding 16.736 J/g while fats yield more than double that (37.656 J/g, Sánchez-Peña et al. 2017). Metabolic water, the water produced through catabolism of

carbohydrates, fats, or proteins (Schmidt-Nielsen and Adolph 1964; Frank 1988; Orr et al. 2015) also varies dramatically. The oxidation of carbohydrates yields 0.60 g of metabolic water per gram, and fats yield 1.07 g per gram due to greater oxygen requirements during lipid metabolism, while proteins yield the least metabolic water (0.41 g) and even require water loss for excretion of nitrogenous waste (Mellanby 1942; Davidson and Passmore 1963; Schmidt-Nielsen and Adolph 1964).

In environments like deserts, where extrinsic water is limited, preformed (dietary) water and endogenous water production are key to survival. Dietary intake represents the raw material to produce metabolic water. Food catabolism results in water loss through respiration, urination, and fecal production (Schmidt-Nielsen 1975). Although specific dietary composition is often unknown and highly variable, particularly for wild animals, energy potential (Pyke et al. 1977; Pyke 1984), nutrition, and capacity for water production (Schmidt-Nielsen and Adolph 1964) all depend on diet composition. In deserts, vegetation, seeds, and insects comprise the bulk of rodent diets (Reichman 1975) with each varying in fat, protein, and carbohydrate composition and may exhibit seasonal availability (Wolf and del Rio 2003; Orr et al. 2015). In response to natural variation in resource availability, animals have the opportunity to make dietary decisions that may have a substantial impact on their internal water economy.

Mice of the genus *Peromyscus* have the widest distribution of any North American mammal and are considered model organisms in evolutionary biology due to their unparalleled habitat diversity (Bedford and Hoekstra 2015), extensive genomic resources (*e.g.,* Tigano et al. 2020; Colella et al. 2021), abundance of historically and contemporaneously collected samples in natural history museums (Pergams and Lawler

2009; Pardi et al. 2020), and ability to live and breed under laboratory conditions (Crossland et al. 2014). The desert specialist cactus mouse, *Peromyscus eremicus*, is endemic to the southwestern United States and exhibits behavioral (Murie 1961; Veal and Caire 1979), physiological (Macmillen 1965; Kordonowy et al. 2017; Colella et al. 2021a), and molecular (MacManes 2017; Tigano et al. 2020) adaptations to desert environments, making it an interesting natural experimental model to examine the potential for adaptation to warmer, drier environments. *Peromyscus eremicus* are omnivorous and opportunistic in their diet, utilizing seeds, arthropods, and green vegetation seasonally (Bradley and Mauer 1973; Meserve 1976) and they shift to consuming cactus seeds and/or fruit pulp during the summer months (Orr et al. 2015).

We examine the effect of dietary fat content on the metabolic physiology of the desert-adapted cactus mouse. To accomplish this, we extend previously characterized circadian metabolic patterns for males and females (Colella et al. 2021a) to rates of metabolism and water loss in animals fed an experimental diet low in fat, but unchanged in terms of energy composition and level of macronutrients. This allows us to test the relationships between dietary fats and energy expenditure and water balance, both of which have direct connections to fitness. Given that fats yield more water and energy compared to carbohydrates and proteins, we hypothesized that animals fed an LFD would perform more poorly than those on the SD.

Methods

Animal Care and Experimental Model

We worked with a captive colony of cactus mice, held at the University of New Hampshire. Captive animal care procedures followed guidelines established by the American Society of Mammologists (Sikes et al., 2016) and were approved by the University of New Hampshire Institutional Animal Care and Use Committee under protocol number 103092.

Sexually mature, non-reproductive healthy adults (n = 28 males, n = 28 females) between three and nine months of age, bred from wild-derived lines maintained at the *Peromyscus* Genetic Stock Center of the University of South Carolina (Columbia, South Carolina, USA) were used in this study. Mice were randomly assigned to one of two feeding groups: a standard diet group (SD - LabDiet® 5015*, 26.101% fat, 19.752% protein, 54.148% carbohydrates, energy 15.02 kJ/g, food quotient [FQ, the theoretical RQ produced by the diet based on macronutrient composition, Westerterp, 1993] 0.89 or a low-fat diet group (LFD - Modified LabDiet® 5015 [5G0Z], 6.6% fat, 22.8% protein, 70.6% carbohydrates, energy 14.31 kJ/g, FQ = 0.92). All food was stored in the desert chamber to match the environmental conditions described below. Animals were housed individually in 9.5L animal chambers inside a larger, environmentally controlled room built to simulate the temperature, humidity, and photoperiodic conditions of the Sonoran Desert (Kordonowy et al. 2017; Colella et al. 2021a). Each animal chamber contained dried cellulose-based bedding, and animals were provided food and water *ad libitum*. Mice were acclimated to the LFD for one month and to experimental cages for 24 hours prior to the beginning of metabolic measurements. Animals were weighed to the nearest tenth of a gram on a digital scale before being housed in an experimental chamber for

four days during metabolic data collection. Animals were then weighed at the end of the experiment.

Environmental temperatures and relative humidity (RH) follow a natural, diurnal pattern. Temperature and RH were fixed at 32°C and 10% RH during the light phase for 11 hours (06:00 to 20:00) and dropped to 24°C and 25% RH during a one-hour transition starting at 20:00, then remained constant during the dark phase for seven hours, before the temperature rises again and the RH falls to light phase temperature during a three-hour transition that starts at 06:00. Photoperiod was regular with 16 hours of light and 8 hours of dark. See Figure 1A for a visual representation of environmental conditions.

Metabolic phenotyping

Metabolic phenotyping data were collected starting at 10:00 using a previously described (Colella et al. 2021a) commercially available respirometry system purchased from Sable Systems International (SSI). Physiological data were collected following the methods outlined in Whitfield et al. (2015) and Colella et al. (2021a). Oxygen consumption (VO_2 ml min^{-1}), carbon dioxide production (VCO_2 ml min^{-1}), and water vapor (WVP, kPa) lost via urine, feces, cutaneous, and respiration were continually measured during 72-hour trials. This was repeated twice for each sex under each diet (SD and LFD), resulting in three-day experiments performed sequentially in eight batches of seven mice (and one empty baseline chamber per three-day experiment) at a time, with batches alternating between females and males for a total of 28 adult males and 28 adult females.

Airstreams were pulled from flow-through chambers at a constant rate of 1600 ml min^{-1} (96 L h^{-1}) by an SSI SS-4 sub-sampler pump (one for each chamber), multiplexed through the SSI MUXSCAN, sub-sampled at 250 ml min^{-1} into a Field Metabolic System (FMS) where water vapor, CO_2, and O_2 were analyzed with no scrubbing. The FMS was zeroed and spanned the sensors between each 72-hour experiment following the SSI Instrument Settings and Calibration manual using dry gas with known concentrations of CO_2 and O_2.

To generate accurate individual measurements, chambers were measured in a pseudorandom order for 120s each, approximately three times every hour. Using methods described in Lighton (2018), we used the most stable 50% of each 120s period to produce a single averaged value for the rate of O_2 consumption (ml min^{-1}), rate of CO_2 production (ml min^{-1}), and rate of water loss (RWL) (mg h^{-1}) per measurement window. Lag time between the analyzers was corrected so that measurements represent the same time periods (Lighton 2018). This procedure results in a highly repeatable set of measurements. VO_2 and VCO_2 were calculated using equations 10.5 and 10.6, respectively, from Lighton (2018). Respiratory quotient (RQ) was calculated as the ratio of VCO_2 to VO_2 (Lighton 2018). Energy expenditure (EE), kJ hr^{-1}, was calculated as in Lighton (2018, eq. 9.15). Response variable measurements that were more than three standard deviations away from the mean, which often represented times when animal care staff entered the room, were considered outliers, and therefore were removed from the downstream analysis.

Electrolytes

At 12:00 Eastern Standard Time (EST), immediately following the conclusion of the experiment, animals were weighed and euthanized with an overdose of inhaled isoflurane and decapitation. 120 μL of trunk blood was collected within one minute of death for serum electrolyte measurement using an Abaxis i-STAT® Alinity machine and CHEM8+ cartridges (Abbott Park, IL, USA, Abbott Point of Care Inc.), and calibrated automatically with each cartridge run. CHEM8+ cartridges measure the concentration of Sodium (Na), Potassium (K), Creatinine (Cr), blood urea nitrogen (BUN), Hematocrit (HCT), Glucose (Glu), and ionized Calcium (iCa), which are expected to vary in response to hydration status and renal function. Finally, using Sodium, Glucose, and BUN, we calculated osmolality using the formula in Rasouli (2016). After assessing for normality, we used a student's two-tailed t-test (stats::t.test) to test for significant ($p < 0.05$) differences in weight and electrolytes within and between the treatments and sexes for each experimental group.

Statistical Analysis

All statistical analyses were conducted in R v 4.0.3 (R Core Team 2020) unless otherwise specified. To perform analyses on the shape of the temporally variable curves, we modeled the effect of time in days, diurnal time, and EE, RQ and/or RWL on EE, RQ, and RWL using Generalized Additive Mixed Models (GAMMs) through the function mgcv::gamm (Lin and Zhang 1999; Wood 2017). To avoid violating the assumption of independence, we used the experimental batches and the mice nested within the experimental batch as random effects. The R function "gamm" then simultaneously estimates the variances of these random effects as well as the treatment

effects, thus implicitly adjusting treatment and covariate effects for the effect of correlated nested groups of measurements. As a result, treatments do not explain differences between individual mice, but rather the average difference between groups of mice, because we were interested in the diet treatment and the effect of sex, as well as in the response over the full 72-hour period and the diurnal cycle. Our models included two fixed effects, diet and sex, and four nonlinear smoothing regression terms: time in days as a nonlinear trend, diurnal cycle as a circular spline function, and two of the three respirometry response variables (EE, RQ, and/or RWL). Using the effective degrees of freedom (edf), we were able to determine the degree of non-linearity of each curve (Wood 2017). To test for physiological differences between mice fed each diet during the light and dark phases, we subsetted data collected during the light phase and dark phase for each response variable. Using the subsetted data, we used GAMMs as described above.

To test for differences in the total volume of water lost for each animal over the course of the experiment, rather than the shape of the curve, we estimated the amount of water lost for each individual for every hour of the experiment and summed those values. These values were then used as the response variable in an ANCOVA (car::anova, Fox and Weisberg 2019). The model consisted of two independent variables, sex and diet, and the interaction between them. Weight at the start of the experiment was used as the covariate. Pairwise comparisons were made using the Tukey separation of adjusted means test (emmeans:: emmeans and emmeans::contrast, Lenth 2021).

Results

Data from 56 sexually mature (but non-reproductive) male and female mice were collected as described above. All mice were subjected to standard animal care procedures before the experiment which included a health assessment conducted by licensed veterinary staff. No health issues were detected by veterinary staff, nor were there significant differences in weight between males and females (mean of 22.2 g [range: 15.40 g - 29.10 g] versus 21.2 g [range: 16.91 g - 29.34 g], respectively; Table 1), or between diet groups.

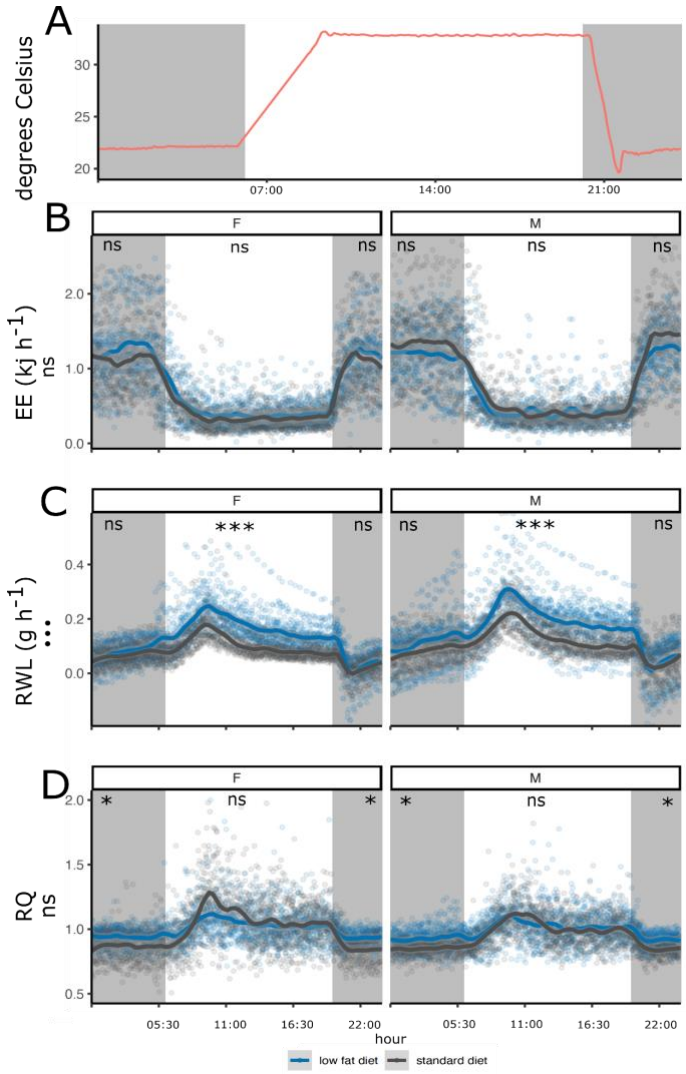

Figure 1

72 hours of room (A) and respirometry (B-D) data collection spit by sex for 28 adult males and 28 adult females plotted in a 24-hour window to display circadian patterns for each group: Organismal responses to standard diet are in gray and low-fat diet in blue. Vertical blocks shaded in gray indicate the dark phase when animals are active, and unshaded blocks indicate light phase when animals are inactive. **A)** Room temperature (℃) is indicated by a solid red line. **B)** 24-hour measurements of energy expenditure (EE kJ hr^{-1}), **C)** rate of water loss (RWL, H_2O g hr^{-1}), and **D)** respiratory quotient (RQ), for females (F, left) and males (M, right). Statistical significance between the fixed effects, diet and sex, for the 24hr GAMMs are denoted with one or more · under the y-axis label, 'ns' indicates non-significance. Statistical significance between the fixed effects, diet and sex, from GAMMs on the subsetted day/night data are denoted with one or more ***** on the top axis of the plots. The two symbols (*/·) signify the same significance values (* p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001).

Electrolytes

Several serum electrolyte values were significantly different between diets for males and females (Sodium p = 0.22 and p = 0.01 respectively; Potassium p = 0.02 and p = 0.01; Blood Urea Nitrogen p = 0.03 and 0.71; Hematocrit p = 0.04 and 0.02; Ionized Calcium p = 0.001 and 0.001; osmolality p = 0.01 and 0.05) (Figure 2, Table1). When comparing males and females on the same diet, Sodium (p = 0.02) and Osmolality (p =

0.03) differed significantly for mice on the SD and no electrolyte measurements were differed between males and females on the LFD.

		Weight (g)	Na (mmol/L)	K (mmol/L)	Cr (µmol/L)	BUN (mmol/L)	Hct (% PCV)	iCa (mmol/L)	Osmolality (mmol/L)
		mean (+/- SD)	mean (+/- SD)	mean (+/- SD)	mean (+/- SD)	mean (+/- SD)	mean (+/- SD)	mean (+/- SD)	mean (+/- SD)
Standard diet	Female	22.4+/-2.73	137.5+/-3.58	8.02+/-1.35	0.22+/-0.04	36.25+/-5.33	26.08+/-9.58	1.1+/-0.1	291.15+/-7.34
	Male	22.24+/-2.14	140.71+/-2.89	7.84+/-0.96	0.2+/-0.03	33.21+/-4.08	32.5+/-2.79	1.16+/-0.07	297.54+/-5.82
	All	21.13+/-2.63	139.23+/-3.56	7.92+/-1.14	0.21+/-0.03	34.62+/-4.85	29.54+/-7.42	1.13+/-0.09	295.04+/-7.1
Low fat diet	Female	20.02+/-2.68	143.6+/-4.7	5.98+/-1.49	0.2+/-0	38.3+/-5.5	34.4+/-3.27	1.29+/-0.07	307.83+/-10.3
	Male	22.29+/-4.05	142.5+/-3.63	6.37+/-1.59	0.2+/-0	37+/-3.53	36.2+/-5.57	1.27+/-0.09	304.18+/-9.22
	All	22.35+/-3.38	143.05+/-4.12	6.18+/-1.51	0.2+/-0	37.65+/-4.55	35.3+/-4.54	1.28+/-0.08	306.01+/-9.7

Table 1

Summary statistics for weight (g) and serum electrolyte measurements (Na = Sodium (mmol/L), K = Potassium (mmol/L), Cr = Creatinine (µmol/L), BUN = Blood Urea Nitrogen (mmol/L), Hct = Hematocrit (% PCV), iCa = Ionized Calcium (mmol/L), and osmolality (mmol/L) for female and male *Peromyscus eremicus* for standard and low-fat diet groups.

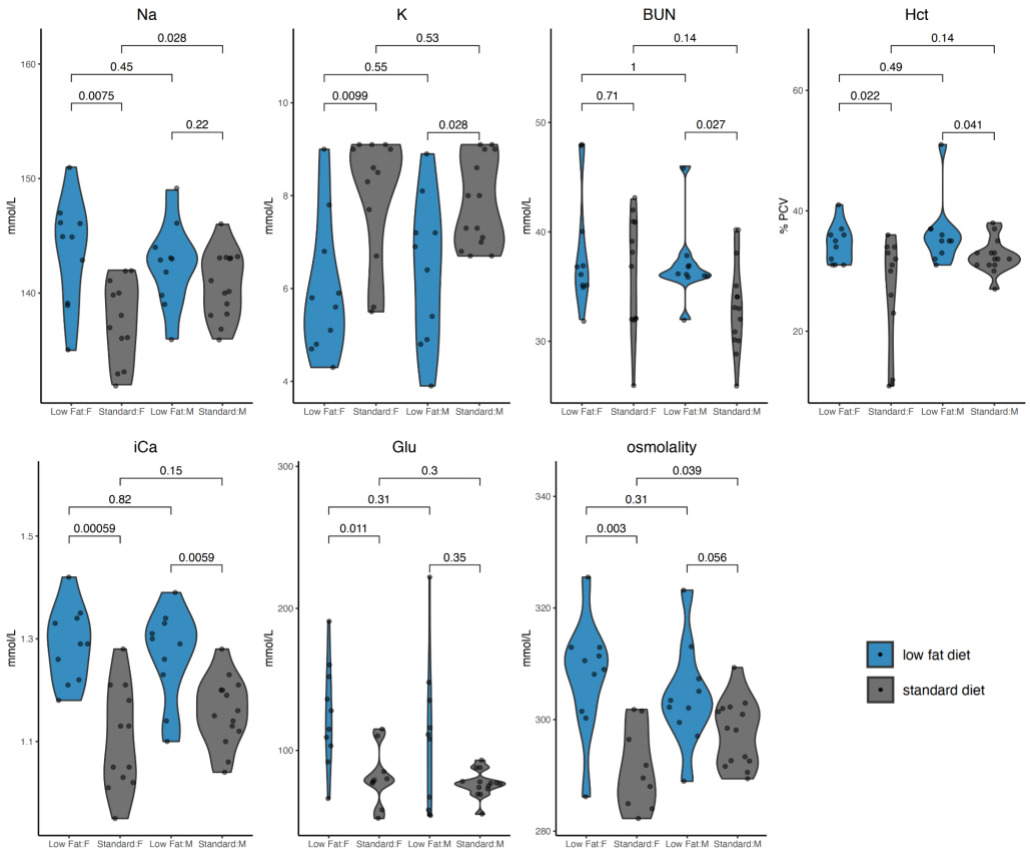

Figure 2

Violin plots showing the distribution of serum electrolyte measurements (Na = sodium (mmol/L), K = potassium (mmol/L), BUN = blood urea nitrogen (mmol/L), Hct = hematocrit (% PCV), iCa = ionized calcium (mmol/L), Glu = glucose (mmol/L), and osmolality (mmol/L) for female (F) and male (M) *Peromyscus eremicus* fed standard (grey) and low fat (blue) diets. Observations (n = 56, 28 for each sex) are represented by black dots. P-values from pairwise t-tests are reported above the brackets.

Metabolic Phenotyping

In total, we recorded 8,125 120s intervals: 4,091 female, 4,034 male (Supplemental Table 1). We measured metabolic variables for 4,105 120s intervals under the LFD:

2,053 female and 2,052 male measurements. For the SD, we recorded 4,020 total observations (2,038 female, 1,982 male). After removing outliers, we retained 3,945 female measurements (1,977 SD and 1,968 LFD) and 3,963 male measurements (1,945 SD, 2,018 LFD). *Peromyscus eremicus* show physiological patterns that are in phase with photoperiod and room temperature for both diets (Figure 1), as described in (Fox and Weisberg 2019; Colella et al., 2021b). All raw data and processed machine-readable csv files are available on Zenodo: https://zenodo.org/record/6422231#.YlXSD9PML0o.

Energy Expenditure

Consistent with previous work (Colella et al. 2021a), males and females for both diets show diurnal patterning of EE, with the highest EE occurring during the dark phase when environmental temperature is lowest, and animals are active. The lowest EE occurred during the light phase, when environmental temperature was greatest and animals were inactive (Figure 1B). Using a GAMM, we modeled EE with diet and sex as fixed effects, as well as other predictors relevant factors such as day, time, RWL, and RQ and found that neither sex nor diet influenced EE (diet $p = 0.56$, sex $p = 0.18$, Supplemental Table 2). When analyzing day and night separately, there were no differences in EE between males and females nor between the two diets during the light or dark phase (light phase: diet $p = 0.550$, sex $p= 0.293$; dark phase: diet $p = 0.571$, sex $p = 0.182$, Supplemental Table 3 and 4). There was a weak nonlinear relationship between EE and day (Supplemental Figure 2A, Supplemental Table 2, edf = 1.98, $p < 2e^{-16}$). Additionally, the relationship between diurnal time and EE was statistically

significant but nonlinear (Supplemental Figure 2F, Supplemental Table 2, edf = 7.98, $p < 2e^{-16}$).

Rate of Water Loss

Both treatment groups, SD and LFD, and both sexes exhibited diurnal patterning of rate of water loss and had the highest rate of water loss during the light phase when environmental temperature is highest, even though animals are inactive (Figure 1C, Supplemental Figure 1F, Supplemental Table 2, edf = 7.95, $p < 2e^{-16}$). Peak loss occurred between 09:00 and 11:00. By fitting a 24-hour time-continuous GAMM (Supplemental Figure 1E-H), we showed that both diet and sex had a significant effect on the RWL (diet $p = 2.13E^{-05}$, sex p = 0.002, Supplemental Table 2), with both males and females fed an LFD losing more water than those fed the SD. When modeling water loss during the light phase only, when animals are inactive, we showed that both diet and sex had a significant effect on RWL (diet $p = 4.80e^{-15}$, sex $p = 1.69e^{-11}$, Supplemental Table 3). When modeling water loss only during the dark phase, when animals are active, RWL was significantly different between males and females (p = 0.015, supplemental table 4), but RWL was not different within sex between different diets (p = 0.797, Supplemental Table 4). The relationship between RWL and experimental day was weakly non-linear, but significant (Supplemental Figure 1E, Supplemental Table 2, edf = 1.99, $p < 2e^{-16}$).

When analyzing the volume of water lost, both diet and sex significantly affect the amount of water lost over the course of the experiment when controlling for individual body weight ($p = 5.22e^{-2}$ and p = 0.017, respectively, Supplemental Table 5), but the

interaction term was not significant (p= 0.633, Supplemental Table 5). Further investigation into the sex:diet interaction showed significant differences in the female diet comparison (p=0.043, Supplemental Table 5), and also in the male diet comparison (p=0.005, Supplemental Table 5).

Respiratory Quotient

RQ showed circadian rhythms for both diets and sexes with the highest RQ occurring during the light phase and the lowest RQ occurring during the dark phase (similar to Colella et al. 2021b; Figure 1D). By fitting a 24-hour GAMM we found no significant difference in RQ between the sexes or the two diets (diet p = 0.52, sex p = 0.83, supplemental 2) but we did find that day and time were highly significant ($p = 1.98e^{-07}$, $p < 2.2e^{-16}$ respectively). Time of day had a complex relationship with RQ (Supplemental Figure 1, edf = 7.69, Supplemental Table 2) and experimental day had a weak non-linear relationship with RQ (Supplemental Figure 1D, edf = 1.59, Supplemental Table 2).

When modeling RQ during the light phase, RQ rose above the FQ, with females fed the SD having the highest RQ at the start of the light phase and then stabilizing at an RQ > 1 around 14:00. We showed that neither sex nor diet were significantly different for RQ (sex p = 0.740, diet p = 0.543, Supplemental Table 3). When modeling RQ during the dark phase, RQ values were comparable to the FQ of the respective diet for both sexes (FQ LFD: 0.92, FQ SD: 0.89) and RQ was significantly different between the diets (p = 0.017, Supplemental Table 4) but not between males and females (p = 0.739, Supplemental Table 4).

Discussion

In light of current rapid climate change (IPCC 2019), understanding how animals respond to environmental change is of paramount importance. Species unable to adapt in place could relocate, but given the magnitude and rate of change and extent of habitat fragmentation, species may be unable to move fast enough or find suitable habitat elsewhere (Loarie et al. 2009; Beever et al. 2010; Sgrò et al. 2011; Henry et al. 2012). The only option left is adaptive evolutionary change, local extirpation, or in more extreme circumstances, extinction. Extreme environments (*e.g.,* high altitude, deep sea, deserts) and the organisms living in them represent natural experimental models for understanding dynamic organismal responses. This research capitalizes on the experimental tractability of a desert specialist rodent to better understand how diet may influence water economy.

We explored the impacts of differences in dietary fat intake and found that mice fed a diet lower in fat lost more water than mice fed a diet higher in fat. In environments where water scarcity is extreme, even small differences in water balance could have substantial impacts on cognitive function and physical performance, and therefore survival and ultimately reproductive success (Boogert et al. 2018). Our results may clarify the physiological consequences of food availability and dietary choices.

Electrolytes

Electrolytes provide information on the overall metabolic state, renal function, and other core physiological functions (Kutscher 1968), including hydration status (Cheuvront et al. 2010) or disease state. Therefore, to further understand the impacts of diet on

physiological function of endotherms in desert environments, we collected serum electrolyte data from male and female cactus mice fed diets with varying levels of fat. We established diet-specific differences for serum Na, K, Cr, BUN, Hct, iCa, and osmolality values for *P. eremicus*. Notably, the synthetic markers of pathological renal impairment, BUN and Cr, were not significantly different between treatments. Previous studies have shown that the electrolyte-water balance is frequently unaffected when desert rodents are water-deprived for long periods of time (Heimeier et al. 2004; Heimeier and Donald 2006; Boumansour et al. 2021), however, other desert rodent studies have demonstrated evidence of dehydration in the absence of exogenous water without renal impairment (Kordonowy et al. 2017; Boumansour et al. 2021).

Interestingly, key electrolytes that are sensitive to hydration status were significantly different between the dietary treatment groups (Figure 2). Serum Na, serum osmolality, and Hct are all expected to be elevated in clinical dehydration and were found to be elevated in animals fed the LFD. Though some of the contrasts were not statistically significant, measurements were consistently higher in both males and females, and this is a strong sign of differences in hydration. These differences are particularly interesting because while animals fed an LFD had higher rates of water loss than those fed the SD, all animals had access to water *ad libitum*. Elevated serum Na, osmolality, and Hct in the context of normal renal function are evidence of dehydration, but dehydration in neuro-intact animals with free access to water is unusual. While dietary fats may be responsible for changes in solute balance (Friedman et al. 2012), typically the relationship is in the opposite direction to what we found (Abdel-Rahman 2010). One possible hypothesis is related to an impairment of the physiological

mechanisms related to thirst. Thirst is a response to changes in the blood chemistry (*i.e.,* increase in osmolality Gilman 1937; Wolf 1950; Heimeier et al. 2004) or volume (Fitzsimons 1961; Stricker 1966) both of which are monitored by the lamina terminalis circumventricular organs (Bourque 2008; Zimmerman et al. 2017). Activation of these brain regions motivates an organism to find water. Previous studies have found that the expression of two genes, *Rxfp1* and *Pdyn* in relevant cell types, correlate with neural activation under osmotic and hypovolemic thirst in lab mice (Pool et al. 2020). Further studies should examine these genes and others in desert organisms to understand the basis of thirst pathways. Regardless of the process, elevated serum Sodium may have important and complex physiological consequences, which may include elevated blood pressure and increased fluid retention, both of which have obvious implications for survival in deserts and deserve future study.

Energy Expenditure

Not all diets provide animals with the same amount of energy per unit mass (Withers 1982). Different macronutrients have different energy potentials, with carbohydrates and proteins yielding 16.7 kJ/g while fats yield 37.7 kJ/g (Sánchez-Peña et al. 2017). Diet composition influences EE (Acheson 1990; Westerterp 2004; Secor 2009; Beale et al. 2018), as do other intrinsic (*i.e.,* body mass, both temperature, evolutionary history, reproductive stage, activity, or energy intake) and extrinsic (*i.e.,* habitat [including environmental temperature], climate, or social environment; Speakman 1997) variables. In wild populations, dietary switches tend to coincide with seasonal changes that affect resource quantity and/or quality (Noble et al. 2019). Previous studies have recorded

cactus mice shifting diet seasonally, consuming arthropods during the winter (Hope and Parmenter 2007), and transitioning to the consumption of cactus seeds and/or fruits during the summer (Hope and Parmenter 2007; Orr et al. 2015). Many plants native to the Sonoran Desert have a fat content comparable to that of the LFD used in this study (5.49 - 9.88% for leaf tissues and branches, McArthur 1994; 0.90- 3.60% for roots and seeds, Castle et al. 2020).

In the current study, both the low-fat and standard diets were designed to have equivalent amounts of energy per gram of weight. We designed the experiment to control for several intrinsic and extrinsic factors that represent sources of variation in EE, but not individual activity, to show that experimental manipulation of diet does not result in differences in EE when food is provided *ad libitum*. However, EE and activity have been shown to be tightly coupled (Garland et al. 2011; Kaiyala et al. 2012), therefore we believe there to be, at most, limited differences in activity given limited differences in EE. For both diets, animals are likely to eat enough to satisfy energy needs, a hypothesis further supported by the fact that there was no net change in weight over the course of the experiment. Consistent with other studies, we recorded higher EE during the dark phase of the 24-hour cycle when animals were active (Colella et al. 2021a), further supporting that metabolic rate is complex and may be modulated by the environment, activity, or other extrinsic/intrinsic factors.

Rate of Water Loss

In environments like deserts, where water limits survival, even small differences in water loss, like those observed here between diets, may have important implications for

survival. Sources of water loss include evaporation, urine, and fecal, the latter two used for processes related to the elimination of waste products resulting from metabolic processes.

Thermoregulation and water loss: Evaporative water loss is one way for organisms to cool themselves as evaporation is accompanied by heat dissipation (*e.g.,* via latent heat of vaporization) and results in a decrease in body temperature and loss of body mass (Porter and Gates 1969). While body temperature was not measured as a part of this study, numerous studies have shown that desert animals reduce respiratory water loss as a major adaptive physiological mechanism for maintaining water balance in deserts (Schmidt-Nielsen and Schmidt-Nielsen 1951; Hart 1971; MacMillen 1972). Indeed, a fine balance must exist between using water for thermoregulation with the use of water for other critical metabolic processes, and with dehydration. Exaggerated use of water for thermoregulation may be harmful when water is limited. Instead, other less water-intensive mechanisms for heat loss such as conduction, and behavioral changes such as estivation and nocturnality to maintain body-temperature homeostasis may be used but are not quantified in this study.

Despite the potential consequences related to limited water supply, desert *Peromyscus* have shown significant capacity for evaporative cooling for the purposes of thermoregulation (Ramirez et al. 2022). In our study, we found that animals fed the LFD had increased water loss compared to animals fed the SD, which has a higher fat concentration (Figure 1C). While this loss may be related to differences in thermoregulatory performance, the increased loss of water may further compromise

water balance. Are these patterns suggestive of differences in physiological performance? Is the consumption of a diet lower in fat related to a reduction in physiological performance and, consequently, fitness? While the current experiment cannot definitively answer these questions, there is some evidence (*e.g.,* electrolyte data) to suggest that physiological performance is impaired in animals consuming the LFD and that this performance, particularly when water is scarce, may have fitness consequences.

Potential mechanisms for increased water loss on LFD: Across all comparisons, there is a strong and statistically significant relationship between the rate of water loss and the fat content of the ingested diet, with individuals fed the LFD losing more water. While there are some potential mechanisms underlying these findings, all of which will require future experiments, several can be ruled out. First, as described above, the maintenance of water balance is a process regulated by a cascade of hormones including arginine vasopressin and the renin–angiotensin–aldosterone system pathway. While some hormones are lipid-based (*e.g.,* aldosterone) and their production and secretion are linked to dietary composition (*i.e*., sex hormones and severe low body fat), the degree to which dietary fats are reduced for animals fed the LFD are not to the level below which hormonal imbalance should be seen. Second, while there is a known relationship between ketogenic diets and dehydration (Wheless 2001; Freeman et al. 2006), these diets are associated with enhanced diuresis. We observed the opposite with animals fed the higher-fat diet losing less water than animals fed the LFD. Third, diets higher in protein require more water for the removal of nitrogenous wastes (Calder

and Braun 1983), these do not account for the observations herein. There is a small difference in the amount of dietary protein (18.9% in SD versus 19.5% in LFD). This small difference is unlikely to result in a difference in water loss and therefore hydration status.

While food and water intake were not measured, a potential mechanism for the observed differences could be that animals fed the LFD simply eat more than animals fed a diet higher in fat. While the diets are closely matched in terms of available energy per unit mass, the LFD is slightly less energy dense. Additionally, it's possible that there are substantial differences in the non-nutritive fraction of the diet that could explain the observations. While the current study does not disentangle this potential aspect, future work could. These differences suggest that animals fed the LFD may be ingesting more food mass to maintain nutritional homeostasis and with food intake increased there may also be increased water intake (Bachmanov et al. 2002). While we have no evidence for this, an increased rate of consumption would potentially result in an increased rate of urine production and the production of water-containing fecal material. Given that the animals fed the LFD do seem to be more dehydrated than animals fed the SD (increased Sodium, osmolarity, HCT, Figure 2), this hypothesis requires that animals fed the LFD are consuming more water, but not enough to satisfy their physiological needs. Given that the animals have free access to water, an unmet need is irreconcilable without further explanation.

Another intriguing hypothesis linked to the concept of unmet hydrational needs has to do with thirst pathways. Thirst is a complex phenotype under tight neural control. In brief, thirst is triggered in response to a reduction in blood volume or an increase in

serum Sodium (Thornton 2010). Osmo- and baroreceptors located in critical parts of the vasculature are responsible for monitoring water balance, and when triggered, set into motion a hormonal cascade that simultaneously enhance renal water retention and stimulate thirst (Thornton 2010; Leib et al. 2016). While the former aspects of the response are crucial to a large number of physiological processes, the latter response, thirst, could be responsible for the observations described above. While purely a hypothesis, there are several examples of species losing parts of their sensory systems via natural selection such as loss of sight (Gore et al., 2018), decreases in certain taste sensitivities or loss of taste entirely (Jiang et al. 2012), and loss of specific olfactory functions (Kishida et al. 2015), resulting in dramatic changes from the habitat in which they have evolved. For animals in deserts where water is largely absent, could well-developed pathways leading to thirst be similarly changed? While requiring careful consideration, perhaps using a comparative genomics approach, this hypothesis could explain why animals with free access to water remain significantly dehydrated.

Respiratory Quotient

RQ, the ratio of CO_2 produced to O_2 consumed, can inform rates of differential fuel utilization. RQ values typically fall between 0.7 and 1.0, with the catabolism of fats yielding 0.7, carbohydrates 1.0, and proteins intermediate values (Kleiber 1975). RQ values exceed 1.0 when anabolism of fat exceeds catabolism (*i.e.,* DNL Benedict 1937; Abreu-Vieira et al. 2015; Levin et al. 2017) or during anaerobic exercise (Whipp 2007; Zagatto et al. 2012). RQ was greater than 1.0 during the inactive period in animals on both low and standard diets suggesting that *de novo* lipogenesis (DNL) is not limited by

fat intake in this species. Here and in previous experiments on *P. eremicus* (Colella et al. 2021a) RQ is only greater than 1.0 during the light phase when animals are inactive, which strongly suggests DNL. While carbohydrates, fats, or proteins are all burned as fuel, if present in excess, they can also be converted to fat which can be stored for future use (Barboza et al. 2009). DNL is one mechanism for converting non-fat energy into a form that can be stored. The conversion of glucose to fats prior to entry into the citric acid cycle is exothermic and results in an energetic cost, representing a metabolically inefficient use of dietary substrate in cold habitats (Solinas et al. 2015), but a potentially efficient mechanism of heat regulation in hot deserts. Further, fat storage could be a way for desert mammals to buffer against an uncertain future where water may be less available, as fat oxidation releases water (Mellanby 1942; Davidson and Passmore 1963; Schmidt-Nielsen and Adolph 1964). Given this, the conversion of other fuel sources to fats may serve as an important reservoir of metabolic water. Previous studies have shown that DNL can occur under high-carbohydrate and high-fat diets (Strable and Ntambi 2010). This is supported by our results, as both groups had an RQ greater than 1.0 during the light phase and RQ values at or below the FQ during the dark phase, which indicates a shift to the supplied dietary substrate as the main oxidative fuel during cooler, dark periods.

Conclusion

Desert-adapted species offer a unique opportunity to study how organisms survive some of the harshest habitats on Earth. The main challenges to survival in desert habitats are maintaining water balance and thermoregulation, with dietary composition

being critically important, particularly to the former. Examining the physiological responses of animals fed diets with different fat content aids in our understanding of how animals may respond to changing environments where food availability and temperatures may change. This will ultimately be important in predicting an animal's response to climate change because diet impacts water balance.

To understand ways in which the desert-adapted cactus mouse, *P. eremicus,* responds to variation in dietary fat content, reproductively mature mice were subjected to one of two diets, an SD or an LFD. In general, EE and RWL for mice on the SD during the light phase (a measurement comparable to the resting metabolic rate) agreed with earlier studies of *Peromyscus* (Withers 1992; Ramirez et al. 2022), although the experimental design presented here more closely represents the diurnal conditions under which animals have evolved. We show that dietary fat is important for water regulation in the desert-adapted cactus mouse through the analysis of interacting physiological traits: 1) high rates of total water loss during the warmer, drier light phase and 2) a shift in serum electrolyte values indicating dehydration. These factors suggest that a low-fat diet could limit the capacity of desert animals to tolerate limited access to free water as is common in arid environments.

Although we were able to collect hundreds of measurements for each diet, sex, and time interval, our work has several limitations. Future studies should further modify the major diet components (carbohydrates and proteins) and assess the seasonal availability of such macronutrients in natural environments. Additionally, the incorporation of food and water intake, activity monitoring, and body temperature will provide further insight into the capacity of *P. eremicus* to tolerate environmental change.

The identification of short-term, physiological responses of *P. eremicus* associated with diet composition demonstrates that adaptations that evolved over longer evolutionary timescales affect survival during the accelerated time scales of climate change. In light of global climate change and increased desertification, investigating the range and mechanisms of plastic response of desert-adapted species with regard to diet composition could provide insights into physiological responses to increasingly erratic climate.

Acknowledgments

We would like to thank members of the MacManes lab for helpful comments and support on previous versions of the manuscript; the Animal Resources Office and veterinary care staff at the University of New Hampshire; A. Gerson at the University of Massachusetts Amherst, Z. Cheviron at the University of Montana, B. Joos and J. Klok at Sable Systems International for analytic guidance, technical support, and respirometry training. This work was supported by the National Institute of Health National Institute of General Medical Sciences (R35 GM128843 to M.D.M.).

Author Contributions

Conceptualization: M.D.M.; Methodology: D.M.B., M.D.M.; Formal analysis: D.M.B., E.L.; Investigation: D.M.B., J.P.C.; Resources: M.D.M.; Writing - original draft: D.M.B.; Writing - review & editing: D.M.B., J.P.C., E.L., M.D.M.; Visualization: D.M.B., E.L.; Supervision: M.D.M.; Project administration: M.D.M.; Funding acquisition: M.D.M.

Competing Interests

No competing interests declared.

Data Availability

Raw ExpeData (SSI) files are available through Zenodo: https://zenodo.org/record/6422231#.YlXSD9PML0o. Macro processing files, raw and processed respirometry data, and cage sampling scheme files are also available on Zenodo. All R scripts used in this project are available through GitHub at: https://github.com/DaniBlumstein/Diet_paper.

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Supplemental

Table S1: Number of samples collected per time interval.

time interval	diet	number of 120 intervals male	number 120 intervals female
light	standard	867	879
	low fat	914	927
dark	standard	758	755
	low fat	758	719

Table S2: Generalized additive mixed models (GAMM) 24-hour results. **A)** Statical model and results for rate of water loss (RWL, H_2O g/hr^{-1}), **B)** model and results for measurements of energy expenditure (EE kJ/hr^{-1}), **C)** model and results for respiratory quotient (RQ).

A)

H2Og~diet+Sex+s(ctime.exp,k=3)+s(time_in_D,bs=cc)+s(EE_kJH)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	0.121	0.008	15.582	<2.00E-16	***
diet	-0.038	0.009	-4.253	0.000	***
Sex	0.027	0.009	3.004	0.003	**
	edf	Ref.df	F	p-value	
s(ctime.exp)	1.992	1.992	183.99	<2e-16	***
s(time_in_D)	7.948	8	668.73	<2e-16	***
s(EE_kJH)	6.565	6.565	20.69	<2e-16	***
s(RQ)	8.422	8.422	37.87	<2e-16	***
R-sq.(adj) = 0.561					
Scale est.=0.0016156	n=7908				

B)

log(EE_kJH)~diet+Sex+s(ctime.exp,k=3)+s(time_in_D,bs=cc)+s(H2Og)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	-0.542	0.063	-8.628	<2e-16	***
diet	-0.043	0.073	-0.585	0.558	
SexM	0.097	0.073	1.332	0.183	
	edf	Ref.df	F	p-value	
s(ctime.exp)	1.980	1.980	39.660	<2e-16	***
s(time_in_D)	7.976	8.000	1464.100	<2e-16	***
s(H2Og)	7.114	7.114	28.930	<2e-16	***
s(RQ)	8.136	8.136	178.830	<2e-16	***
R-sq.(adj)=0.678					
Scale est.=0.076286	n=7908				

C)

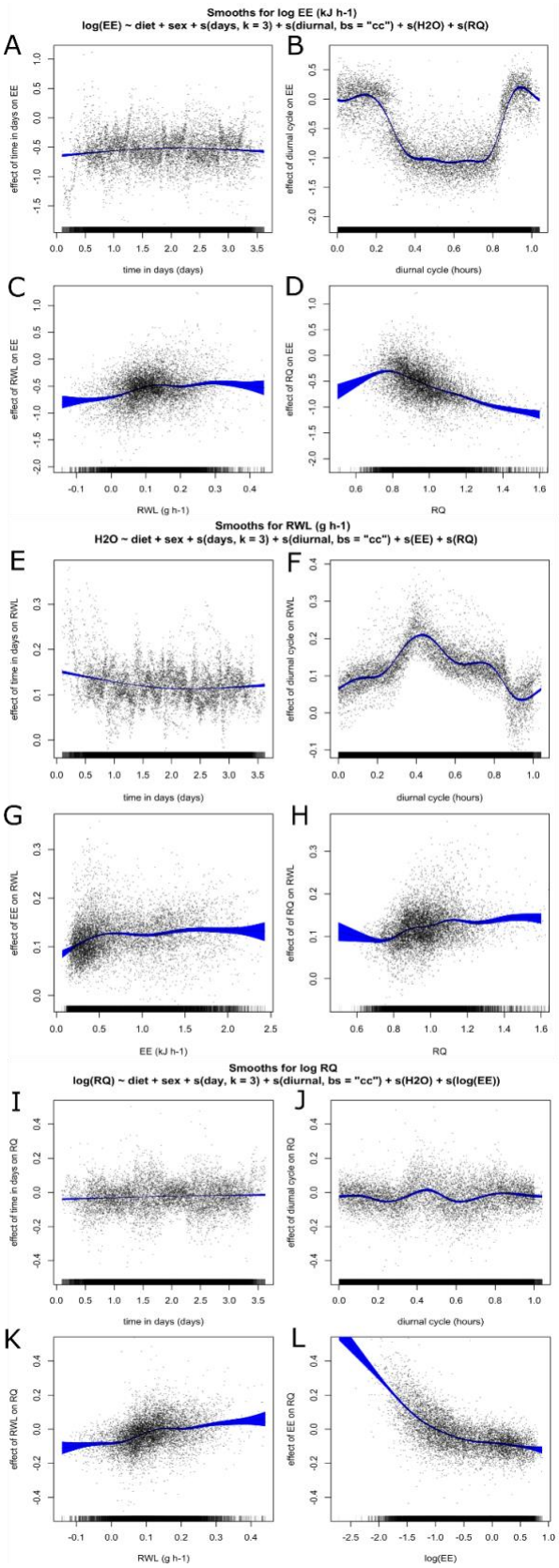
log(RQ)~diet+Sex+s(ctime.exp,k=3)+s(time_in_D,bs=cc)+s(H2Og)+s(log(EE_kJH))

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	-0.024	0.034	-0.706	0.480	
diet	-0.025	0.039	-0.641	0.522	
Sex	-0.008	0.039	-0.210	0.834	

	edf	Ref.df	F	p-value	
s(ctime.exp)	1.593	1.593	27.450	1.98E-07	***
s(time_in_D)	7.693	8.000	44.530	< 2.00E-16	***
s(H2Og)	7.397	7.397	47.360	< 2.00E-16	***
s(log(EE_kJH))	7.160	7.160	359.320	< 2.00E-16	***

R-sq.(adj)=0.393

Scale est.=0.0070625 n=7908


Smooths for log EE (kJ h-1)
log(EE) ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(H2O) + s(RQ)
A
effect of time in days on EE
time in days (days)
B
effect of diurnal cycle on EE
diurnal cycle (hours)
C
effect of RWL on EE
RWL (g h-1)
D
effect of RQ on EE
RQ
Smooths for RWL (g h-1)
H2O ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(EE) + s(RQ)
E
effect of time in days on RWL
time in days (days)
F
effect of diurnal cycle on RWL
diurnal cycle (hours)
G
effect of EE on RWL
EE (kJ h-1)
H
effect of of RQ on RWL
RQ
Smooths for log RQ
log(RQ) ~ diet + sex + s(day, k = 3) + s(diurnal, bs = "cc") + s(H2O) + s(log(EE))
I
effect of time in days on RQ
time in days (days)
J
effect of diurnal cycle on RQ
diurnal cycle (hours)
K
effect of RWL on RQ
RWL (g h-1)
L
effect of EE on RQ
log(EE)

975

Figure S1: Contributions of model terms to energy expenditure (EE kJ h-1, A-D), rate of water loss (RWL g h-1, E-H), and respiratory quotient (RQ, I-L) for female and male *Peromyscus eremicus* across two diet groups (standard diet and low-fat diet) in general additive mixed models (GAMM). The smoothing curves for each response variable included two fixed effects; diet (low fat vs standard) and sex, two random effects; mouse identification number and date of data collection, and four regression terms: time in days, diurnal cycle, and two of the three respirometry response variables (EE, RQ, and/or EWL) as regression terms. For each graph, the y-axis is the effect of the x-axis on the respirometry response variable estimated by a multivariable GAMM. Blue-shaded areas are 95% confidence intervals, black points are residuals.

Table S3: Generalized additive mixed models (GAMM) results for only data collected during the light phase. A) Statical model and results for rate of water loss (RWL, H2O g/hr-1), B) model and results for measurements of energy expenditure (EE kJ/hr-1), C) model and results for respiratory quotient (RQ).

A)
H2Og~diet+Sex+s(ctime.exp,k=3)+s(EE_kJH)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	0.15503	0.00756	20.506	<2.00E-16	***
diet	-0.058941	0.008737	-6.746	1.69E-11	***
Sex	0.035545	0.008736	4.069	4.80E-05	***

	edf	Ref.df	F	p-value	
s(ctime.exp)	1.974	1.974	68.25	<2e-16	***
s(EE_kJH)	5.797	5.797	10.8	<2e-16	***
s(RQ)	5.309	5.309	102.25	<2e-16	***

R-sq.(adj)=0.349
Scale est.=0.0023669 n=4918

B)
log(EE_kJH)~diet+Sex+s(ctime.exp,k=3)+s(H2Og)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	-0.92542	0.09277	-9.975	<2e-16	***
diet	-0.06415	0.10738	-0.597	0.55	
Sex	0.11275	0.10719	1.052	0.293	

	edf	Ref.df	F	p-value	
s(ctime.exp)	1	1	6.991	0.00822	**
s(H2Og)	6.797	6.797	7.118	<2.00E-16	***
s(RQ)	5.875	5.875	285.938	<2.00E-16	***

R-sq.(adj)=0.189
Scale est.=0.12779 n=4918

B)
log(RQ)~diet+Sex+s(ctime.exp,k=3)+s(H2Og)+s(log(EE_kJH))
Parametric coefficients:

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	0.001688	0.036754	0.046	0.963	

diet	0.025869	0.042478	0.609	0.543	
Sex	-0.014092	0.042453	-0.332	0.74	
	edf	Ref.df	F	p-value	
s(ctime.exp)	1.43	1.43	18.81	1.27E-05	***
s(H2Og)	7.305	7.305	91.83	<2.00E-16	***
s(log(EE_kJH))	6.539	6.539	362.65	<2.00E-16	***
R-sq.(adj)=0.283					
Scale est.=0.0084596	n=4918				

Table S4: Generalized additive mixed models (GAMM) results for only data collected during the dark phase. A) Statical model and results for rate of water loss (RWL, H2O g/hr-1), B) model and results for measurements of energy expenditure (EE kJ/hr-1), C) model and results for respiratory quotient (RQ).

A)

H2Og~diet+Sex+s(ctime.exp,k=3)+s(EE_kJH)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	0.053382	0.00923	5.784	8.07E-09	***
diet	0.002749	0.01071	0.257	0.797	
Sex	0.025882	0.010637	2.433	0.015	*

	edf	Ref.df	F	p-value	
s(ctime.exp)	1.968	1.968	19.063	4.29E-07	***
s(EE_kJH)	4.881	4.881	8.185	6.41E-07	***
s(RQ)	7.887	7.887	47.955	<2.00E-16	***

R-sq.(adj)=0.219

Scale est.=0.0018757 n=2990

B)

log(EE_kJH)~experiment+Sex+s(ctime.exp,k=3)+s(H2Og)+s(RQ)

	Estimate	Std.	Error	t value	Pr(>|t|)
(Intercept)	0.08701	0.07403	1.175	0.24	
diet	-0.04856	0.0857	-0.567	0.571	
Sex	0.11419	0.08547	1.336	0.182	

	edf	Ref.df	F	p-value	
s(ctime.exp)	1.97	1.97	21.35	<2e-16	***
s(H2Og)	7.853	7.853	12.85	<2e-16	***
s(RQ)	7.469	7.469	34.63	<2e-16	***

R-sq.(adj)=0.12

Scale est.=0.058025 n=2990

C)

log(RQ)~experiment+Sex+s(ctime.exp,k=3)+s(H2Og)+s(log(EE_kJH))

Parametric coefficients:

	Estimate	Std.	Error	t value

(Intercept)	-0.07254	0.02994	-2.423	0.0155	*
diet	-0.08263	0.03458	-2.39	0.0169	*
Sex	-0.01154	0.03459	-0.333	0.7388	

	edf	Ref.df	F	p-value	
s(ctime.exp)	1.95	1.95	20.1	<2e-16	***
s(H2Og)	6.454	6.454	45.5	<2e-16	***
s(log(EE_kJH))	1	1	39.76	<2e-16	***

R-sq.(adj)=0.292

Scale est.=0.0032848 n=2990

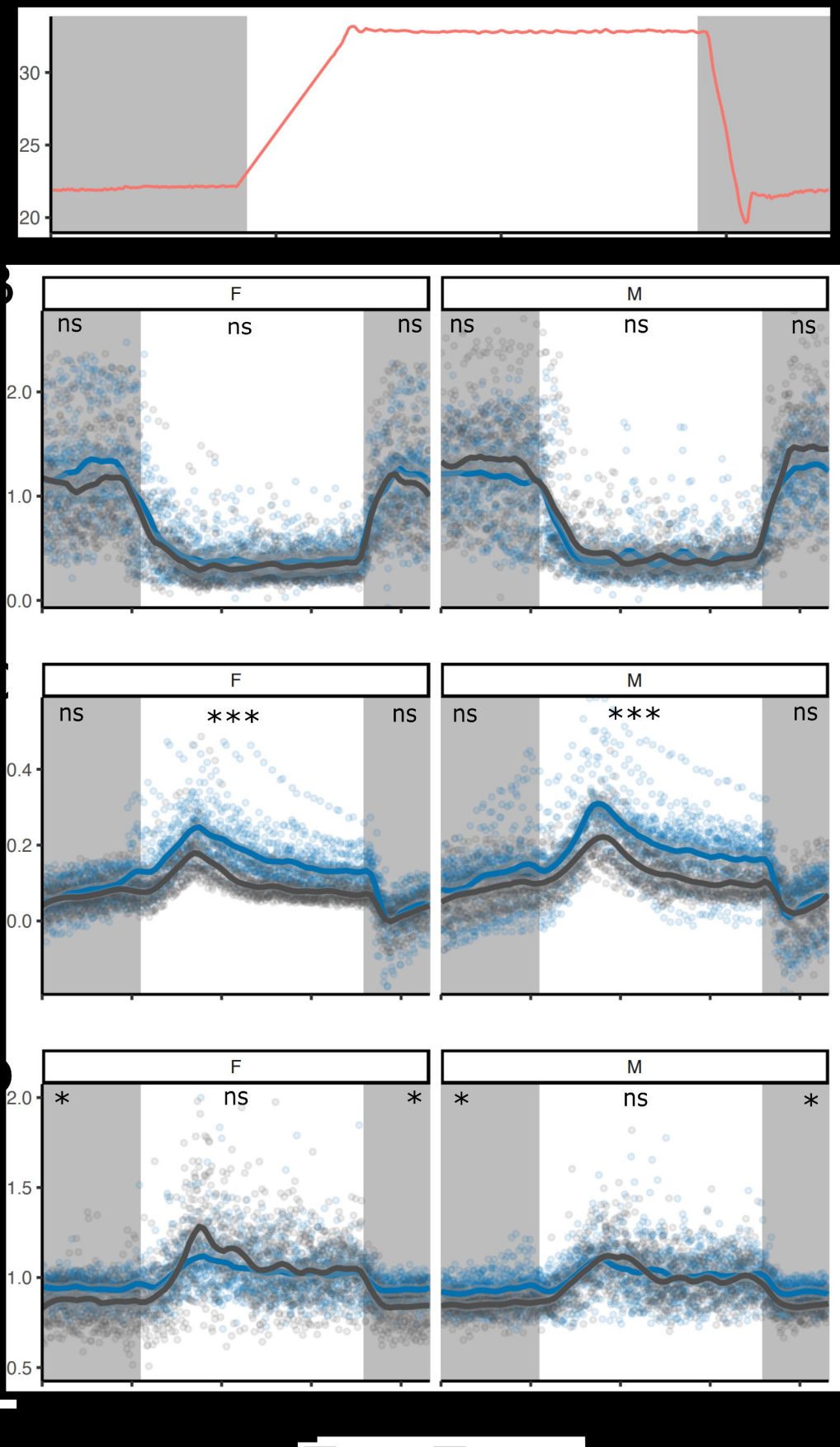
999

1000

Table S5: ANCOVA results for total water loss.

	Sum	Sq	Df	F value	Pr(>F)
diet	130.36	1	19.5126	5.22E-05	***
sex	40.4	1	6.0471	0.01736	*
weight	7.03	1	1.0529	0.30967	
diet:sex	1.54	1	0.2305	0.63317	
Residuals	340.72	51			

	estimate	SE	df	t.ratio	p.value
contrast					
F Low Fat - M Low Fat	-2.05	0.977	51	-2.101	0.1667
F Low Fat - F Standard	2.76	1.018	51	2.716	0.0433
F Low Fat - M Standard	1.39	0.977	51	1.421	0.4922
M Low Fat - F Standard	4.82	1.014	51	4.748	0.0001
M Low Fat - M Standard	3.44	0.977	51	3.522	0.0049
F Standard - M Standard	-1.38	1.013	51	-1.359	0.5307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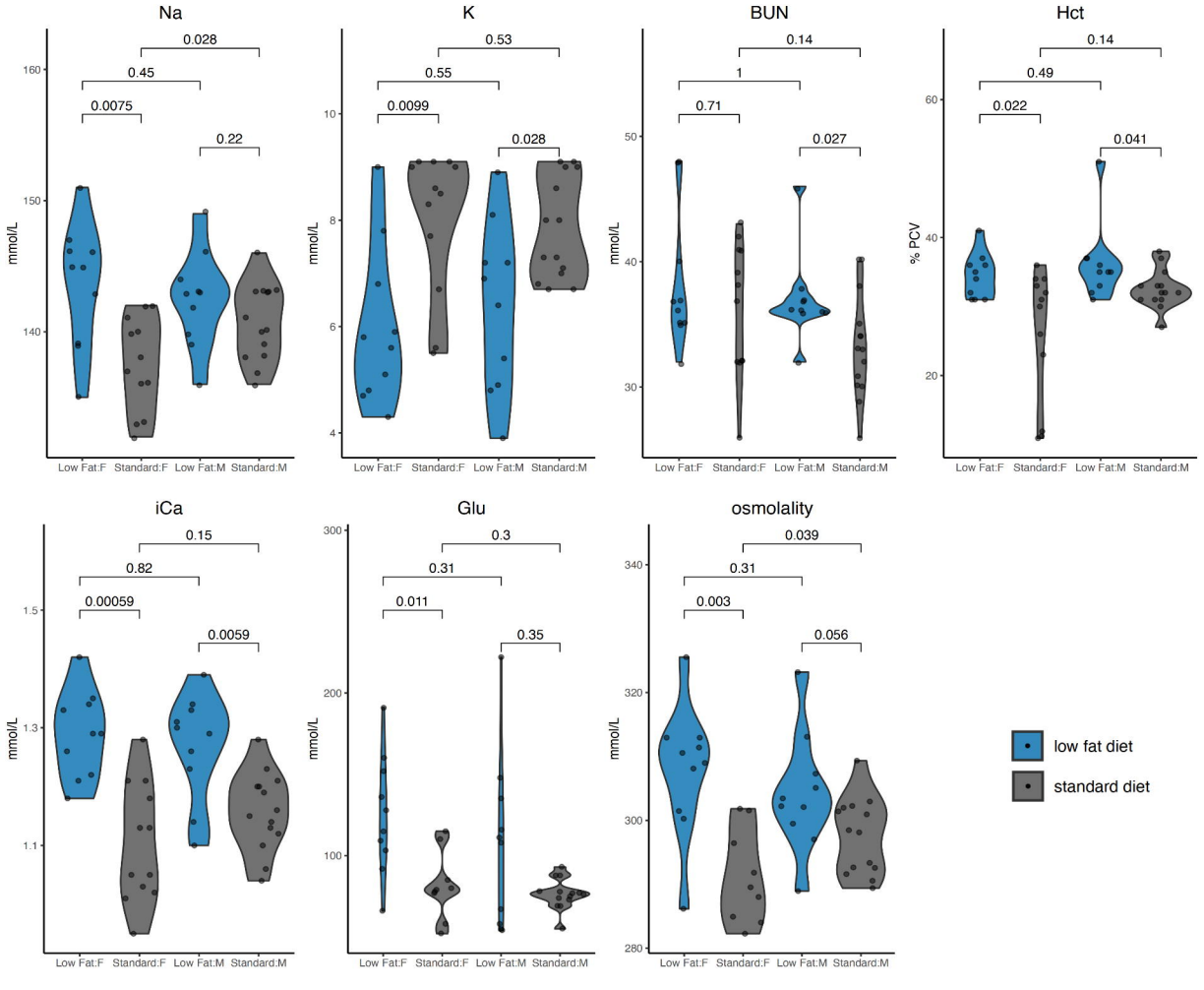
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50
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mmol/L
Hct
0.14
0.49
0.022
0.041
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20
% PCV
iCa
0.15
0.82
0.00059
0.0059
1.5
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mmol/L
Glu
0.3
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low fat diet
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