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

Danielle M. Blumstein[1], Jocelyn P. Colella[1,2], Ernst Linder[3], Matthew D. MacManes[1]

1. University of New Hampshire, Molecular, Cellular, and Biomedical Sciences Department, Durham, NH 03824 (DMB Dani.Blumstein@unh.edu, Orcid ID: 0000-0003-2129-5541; MDM Matthew.MacManes@unh.edu, Orcid ID: 0000-0002-2368-6960)

2. University of Kansas, Biodiversity Institute, Lawrence, KS 66045 (JPC colella@ku.edu, Orcid ID: 0000-0003-2463-1029)

3. University of New Hampshire, Department of Mathematics and Statistics, Durham, NH 03824 (EL ernst.linder@unh.edu)

Abstract

Availability of essential food resources is one of the most important drivers of survival and to persist in environments where critical resource abundance is changing, animals must either relocate or adapt in place. Testing an animal's ability to respond to alternative conditions can reveal differences in physiological responses. 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 Sonoran Desert and treated mice with two different diets; a standard diet and a low-fat diet. We found significant diet-specific differences in the rate of water loss and serum electrolyte values. Mice fed the low-fat diet lost more water relative to those eating the standard diet and patterns of *de novo* lipogenesis are not limited by dietary

composition. Our results are consistent with the hypothesis that diet contributes to water homeostasis. Compared to other desert animals, rodents have limited capacity to dissipate heat using evaporative cooling, limiting thermoregulatory performance at higher temperatures. We predict that a mismatch in physiological requirements and environmental conditions could significantly impact small mammal survival in desert environments if optimal foods become less abundant, as may be the case given climate change.

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

Introduction

As the planet undergoes unprecedented climate change, increased desertification is predicted (IPCC, 2019; Mirzabaev et al., 2019; Wu et al., 2014). Indeed, water limitation is a strong selective force that will result in changes to species distributions and community composition. Understanding these responses is critically important for predicting the impacts of rapid climate change on biodiversity. Responses to climate change vary but can be classified into two general categories: geographic relocation to maintain physiological balance (Moritz et al., 2008; Riddell et al., 2021; Walsh et al., 2016) or, for species with fragmented habitats or limited dispersal ability, adaptation in place (Reed et al., 2011). With regards to the former, several studies have demonstrated or predicted distributional changes of species in response to changing climate (Moritz et al., 2008; Morrison et al., 2018; Parmesan, 2006; Parmesan and

Yohe, 2003; Pecl et al., 2017; Van de Peer et al., 2017). The latter response is less well documented. Adaptation *in situ* may involve morphological, behavioral, or physiological changes, such as directional selection for adaptive seasonal camouflage (Jones et al., 2018), but in the absence of genetic variation, there is strong evidence of increased extinction risk (Spielman et al., 2004).

The Sonoran Desert is hot, dry, and dynamic, temperatures fluctuate by >15℃ within a single day and >30℃ between seasons, and average relative humidity changes by 20% between seasons (Reid, 1987; Sheppard et al., 2002; Unland et al., 1996). High ambient temperatures and lack of extrinsic water challenges mammalian survival, as most mammals rely on 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 (Bittner et al., 2021; Bradford, 1974; Cortés et al., 2000; Kordonowy et al., 2017; MacManes, 2017; MacMillen and Hinds, 1983; Nagy, 1987; Tigano et al., 2020)?

Animals that have evolved in desert environments are physiologically adapted to extreme temperatures and aridity, and therefore represent an interesting natural experimental model to examine adaptation to these conditions, and the potential for adaptation given climate change. 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), seeking microhabitat refugia during the heat of the day (Riddell et al., 2021), and nocturnal habits (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 adaptation, such as efficient kidney function or low fecal water content which are observed in desert mammals to maintain water balance, occur across evolutionary time scales (Christian and Matson, 1978; Kordonowy and MacManes, 2016). Genomic studies have found evidence of positive selection in desert mammals, acting on genes involved in energy production, food storage (Jirimutu et al., 2012; Wu et al., 2014), water reabsorption (Giorello et al., 2018; Jirimutu et al., 2012; Marra et al., 2012; Marra et al., 2014; Yang et al., 2016), and osmoregulation (Colella et al., 2021a; Kordonowy and MacManes, 2016; MacManes and Eisen, 2014). A strong signature of positive selection on Growth Differentiation Factor 10 (*Gdf10*) has also been found and may prevent weight gain under high-fat diets (Hino et al., 2017; Tigano et al., 2020). While this prior research has enhanced our understanding of the physiologic processes underlying adaptation in desert environments, we still don't fully understand the role that diet plays in water economy regulation.

In environments like deserts, where extrinsic water is limited, endogenous water production is likely critically important to survival, and dietary intake represents the raw material for the production of metabolic water (Frank, 1988; Schmidt-Nielsen and Adolph, 1964). Metabolic water is the water produced through catabolism of carbohydrates, fats, or proteins. In deserts, insects, vegetation, and seeds comprise the bulk of rodent diets (Reichman, 1975). Although specific dietary composition is often unknown, an optimal diet balances energy gain (Pyke, 1984; Pyke et al., 1977),

nutrition, and capacity for water regulation (Schmidt-Nielsen and Adolph, 1964). Dietary items exhibit seasonal availability in nature, which if consumed in proportion to availability can influence water homeostasis (Orr et al., 2015; Wolf and del Rio, 2003). Additionally, with regards to diet, water potential varies dramatically: oxidation of carbohydrates yields 0.60g of metabolic water per gram, however, fats yield 1.07g per gram due to greater oxygen requirements, while proteins yield the least metabolic water (0.41g) and even require water loss for excretion of nitrogenous waste (Davidson and Passmore, 1963; Mellanby, 1942; Schmidt-Nielsen and Adolph, 1964). We hypothesize that a standard diet will maximize water conservation when compared to a low-fat diet.

Mice of the genus *Peromyscus* have the widest distribution of any North American mammal and are considered a model organism 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. 2021a), an abundance of historically and contemporaneously collected samples in natural history museums (Pardi et al., 2020; Pergams and Lawler, 2009), 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 (Colella et al., 2021b; Kordonowy et al., 2017; Macmillen, 1965), and molecular (MacManes, 2017) adaptations to desert environments. *Peromyscus eremicus* are omnivorous and opportunistic in their diet, utilizing seeds, arthropods, and green vegetation seasonally (Bradley and Mauer, 1973; Meserve, 1976) and shift to intense cactus consumption during the summer months (Orr et al., 2015).

Given the relationship between dietary composition and metabolic water production, the current study examines the effects 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., 2021b) to rates of metabolism and water loss in animals fed an experimental diet low in fat, but otherwise unchanged. Thereby testing the role that dietary fat has on water balance, which has a direct connection to fitness.

Methods

Animal Care and Experimental Model

Animal care procedures followed guidelines established by the American Society of Mammalogists (Sikes and Gannon, 2011; 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, food quotient [FQ] 0.89, supplemental file 3) or a low-fat diet group (LFD - Modified LabDiet® 5015 [5G0Z], 6.6% fat, 22.8% protein, 70.6% carbohydrates, FQ = 0.92, supplemental file 4). Animals were housed individually in 9.5L animal chambers inside a larger, walk-in environmental chamber built to replicate

temperature, humidity, and photoperiodic conditions of the Sonoran Desert (Colella et al., 2021c; Kordonowy et al., 2017). 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. Diurnal temperature and relative humidity (RH) fluctuation are fixed at 32°C and 10% RH during the light phase for 11 hours (06:00 hr to 20:00 hr), and drop to 24°C and 25% RH during a 1 hr transition (T1) starting at 20:00 hr, then remains 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 (T2) that starts at 06:00 hr. Photoperiod was constant: 16 hr light, 8 hr dark (Figure 1). Animals were weighed (g) on a digital scale before being housed in experimental cages for three days during metabolic data collection and weighed again at the end of the experiment.

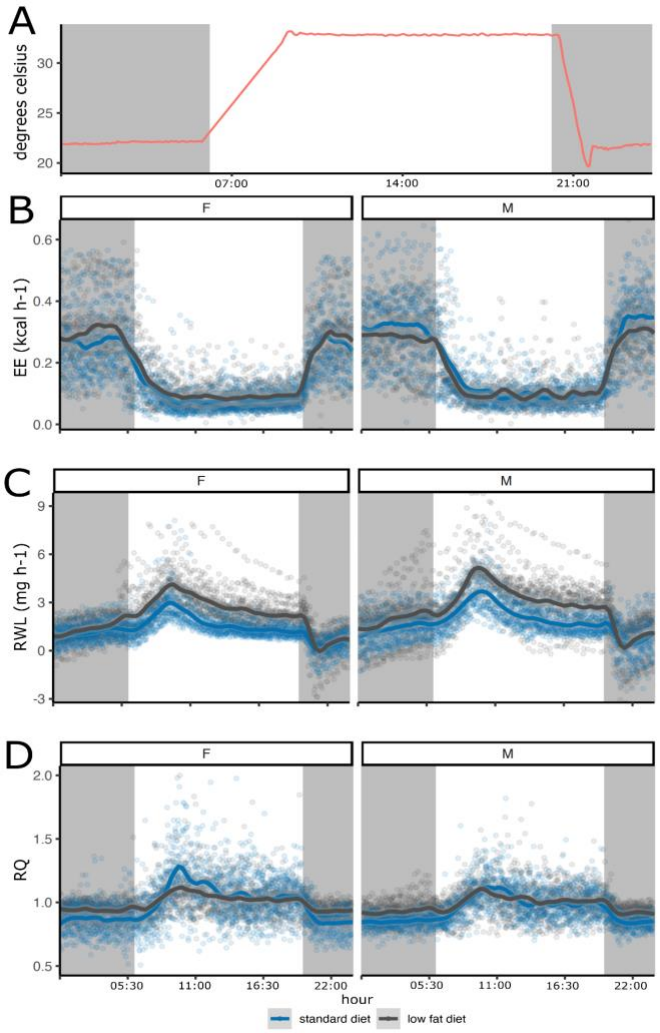

Figure 1

72 hours of data collection plotted in a 24-hour window to display circadian patterns for each group: organismal responses to standard diet are in blue and low-fat diet in gray. Vertical blocks shaded in gray indicate the dark phase, and unshaded blocks indicate light phase. A) Temperature (℃) is indicated by a solid red line. Transition 1 (T1) occurred during the first shift from dark to light at 07:00. Transition 2 (T2) occurred during the second shift from light to dark at 20:00. B) 24-hour measurements of energy expenditure (EE kcal/hr^{-1}), C) rate of water loss (H_2O mg/hr^{-1}), and D) respiratory quotient (RQ), for females (F, left) and males (M, right).

Metabolic phenotyping

Metabolic phenotyping data were collected using a previously described (Colella et al., 2021b) commercially available respirometry system purchased from Sable Systems International. Physiological data were collected following the methods outlined in Whitfield et al. (2015) and Colella et al. (2021b). Oxygen consumption (VO_2 ml min^{-1}), carbon dioxide production (VCO_2 ml min^{-1}), and water vapor (WVP, kPa) were continually measured during 72 hr 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.

Each cage was measured for 120s approximately twice every hour. We used the most stable 50% of each measurement period to produce a single averaged VO_2 (ml min^{-1}), VCO_2 (ml min^{-1}), and rate of water loss (RWL) (mg h^{-1}) value for that interval. 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), kcal/hr^{-1}, was calculated as in Lighton (2018, eq. 9.15). Values for each response variable that were more than three standard deviations away from the mean, often representing times when animal care staff entered the chamber, were considered outliers and removed from the downstream analysis.

Statistical Analysis

All statistical analyses were conducted in R v 4.0.3 (R Core Team, 2020), unless otherwise specified. Source code is available at https://github.com/DaniBlumstein/Diet_paper. We used Generalized Additive Mixed Models (mgcv::gamm, Lin and Zhang, 1999; Wood, 2017) to model the effect of time in days, diurnal time, and EE, RQ and/or RWL on EE, RQ, and RWL. 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 are not explaining differences between individual mice, but rather the average differences between groups of mice because we were interested in the diet treatment and the effect of sex, as well as time traces over the full 72 hr 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) as regression terms.

Electrolytes

At 12:00 EST, immediately following the conclusion of the experiment, animals were weighed and euthanized following approved IACUC protocols. 120 uL of trunk blood was collected within 1 minute of death for serum electrolyte measurement using an Abaxis i-STAT® Alinity machine with the i-STAT CHEM8+ cartridge (Abbott Park, IL,

USA, Abbott Point of Care Inc). CHEM8+ cartridges measure the concentration of sodium, potassium, creatinine, blood urea nitrogen, hematocrit, and ionized calcium, which are expected to vary in response to hydration status and renal function. We used a student's two-tailed t-test (stats::t.test) to test for significant ($p < 0.05$) differences in weight after the treatments were applied and electrolytes between the sexes for each experimental group.

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 and weighing the animals. No significant difference in weight between males and females was detected (mean of 22.2g versus 21.2g, respectively; Table 1), nor between diet groups. No animals were removed from the study due to health concerns.

		females			males		
		all	standard diet	low fat diet	all	standard diet	low fat diet
weight	median	21.94	22.35	21.79	21.55	22.55	19.90
	mean	22.35	22.29	22.40	21.13	22.24	20.02
	range	15.40-29.10	15.40-25.20	17.23-29.34	16.91-29.34	18.20-26.60	16.91-29.34
Na	median	143.00	143.00	145.00	140.00	140.50	137.50
	mean	143.05	142.50	143.60	139.23	140.71	137.50
	range	132-151	132-142	135-151	136-149	136-146	136-149
K	median	5.85	6.65	5.70	8.15	7.65	8.55
	mean	6.18	6.37	5.98	7.90	7.82	7.99
	range	4.30-9.00	5.50-9.00	4.30-9.00	3.90-9.00	6.70-9.00	3.90-8.90
Cr	median	0.20	0.20	0.20	0.20	0.20	0.20
	mean	0.20	0.20	0.20	0.21	0.20	0.22
	range	0.20-0.30	0.20-0.30	0.20-0.20	0.19-0.30	0.19-0.30	0.20-0.20
BUN	median	36.00	36.00	36.50	33.50	33.00	37.50
	mean	37.65	37.00	38.30	34.62	33.21	36.25
	range	26-48	26-43	32-48	26-46	26-40	32-46
Hct	median	35.00	35.00	34.50	32.00	32.00	32.50
	mean	35.30	36.20	34.40	32.08	32.50	31.58
	range	23-41	23-36	31-41	27-51	27-38	31-51
iCa	median	1.29	1.30	1.29	1.14	1.16	1.09
	mean	1.28	1.27	1.29	1.13	1.16	1.10
	range	0.95-1.42	0.95-1.28	1.18-1.42	1.04-1.39	1.041.28	1.10-1.39

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Table 1

Summary statistics for weight (g) and serum electrolyte measurements (Na = sodium, K = potassium, Cr = creatinine, BUN = blood urea nitrogen, Hct = hematocrit, iCa = ionized calcium) for female and male *Peromyscus eremicus* across each diet group, standard and low fat.

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 circadian metabolism in phase with photoperiod and room temperature for both diets (Figure 1), as described in Colella et al. (2021b). All raw data (Expedata files) and processed machine-readable csv files are available on Zenodo: https://zenodo.org/record/6422231#.YlXSD9PML0o.

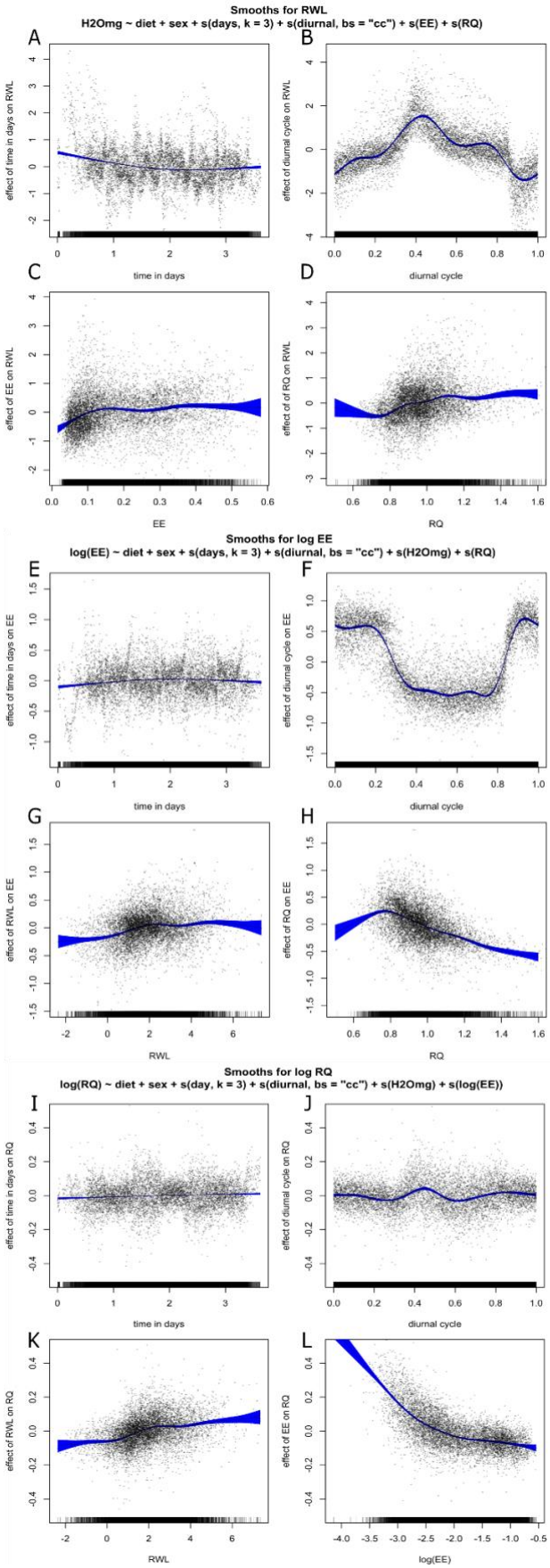
Smooths for RWL
H2Omg ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(EE) + s(RQ)
A
effect of time in days on RWL
time in days
B
effect of diurnal cycle on RWL
diurnal cycle
C
effect of EE on RWL
EE
D
effect of of RQ on RWL
RQ
Smooths for log EE
log(EE) ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(RQ)
E
effect of time in days on EE
time in days
F
effect of diurnal cycle on EE
diurnal cycle
G
effect of RWL on EE
RWL
H
effect of RQ on EE
RQ
Smooths for log RQ
log(RQ) ~ diet + sex + s(day, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(log(EE))
I
effect of time in days on RQ
time in days
J
effect of diurnal cycle on RQ
diurnal cycle
K
effect of RWL on RQ
RWL
L
effect of EE on RQ
log(EE)

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Figure 2

Contributions of model terms to rate of water loss (RWL, A-D), energy expenditure (EE, E-H), and C) 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 bands, black points are residuals.

Rate of Water Loss

Both groups of mice, SD and LFD, and both sexes exhibited diurnal patterning of water loss and had the highest water loss during the light phase (Figure 1C), with peak loss occurring between 09:00 and 11:00. During the second transition (light to dark) water loss dropped sharply until 22:00 hours, before slowly increasing until the first transition (dark to light). Overall, there were substantial differences in the amount of water lost between males and females (male RWL mean: -2.37 - 7.35, female RWL mean: 1.56 - 5.66). For both males and females, mice on the low-fat diet lost more water during the light phase (females LFD mean: 2.74, females SD mean: 1.61, males LFD mean: 3.32, males SD mean: 2.18). Both diet and sex have a significant effect on RWL (diet $p = 1.70e-05$, sex $p = 0.003$, supplemental table 2). Time in days has a weakly negative

relationship with RWL even though it is highly significant (Figure 2A, supplemental 2). The strongest relationship is seen between diurnal time and RWL (Figure 2B, supplemental 2). Finally, EE and RQ, while highly significant, have a weak relationship with RWL (Figure 2C, Figure 2D, supplemental table 2).

Energy Expenditure

Males and females for both diets show diurnal patterning of EE with the highest EE occurring during the dark phase and the lowest EE during the light phase (Figure 1B). Interestingly, males and females had different EE measurements during the dark phase for each diet with females on the SD having higher EE (SD mean: 0.24, LFD mean: 0.28) and males on the LFD diet having higher EE (SD mean: 0.31, LFD mean: 0.29). During the light phase, EE measurements stabilized to similar measurements for all groups (females LFD mean: 0.09 females SD mean: 0.09, males LFD mean: 0.09, males SD mean: 0.09). There was no significant difference in EE between the sexes and the two diets (diet $p = 0.56$, sex $p = 0.18$, supplemental table 2). While significant, time in days had no relationship with EE (Figure 2E, supplemental table 2). The relationship that is also statically significant is seen between diurnal time and EE (Figure 2F, supplemental 2). Finally, RWL had a weak positive relationship with EE and RQ had a negative relationship with EE (Figure 2G, Figure 2H, supplemental table 2).

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. During the

dark phase, RQ values were comparable to the food quotient (FQ) of the diet for both sexes (LFD males mean: 0.92, LFD females mean: 0.94, SD males mean: 0.85, SD females mean: 0.86, FQ LFD: 0.92, FQ SD: 0.89). During T1, RQ increased above the FQ for both diets and sexes, with females on the SD having the highest RQ at the start of the light phase and then stabilizing at 14:00 hours for the remainder of the light phase (LFD males mean: 1.03, LFD females mean: 1.04, SD males mean: 1.03, SD females mean: 1.08). We found no significant difference in RQ between the sexes and the two diets (diet $p = 0.84$, sex $p = 0.52$, supplemental 2). While time in days, diurnal time, RWL, and EE were highly significant, only EE has a weak relationship with RQ (Figure 2I-L, supplemental table 2).

Electrolytes

Serum electrolyte levels did not differ between males and females except for creatine (males/females creatinine 0.21/0.20 $p < 0.05$, Table 1). Between experimental groups, several serum electrolytes were significantly different (SD/LFD sodium 139.23/143.05 $p < 0.05$, potassium 7.90/6.18 $p < 0.05$, creatinine 0.21/0.20 $p < 0.05$, blood urea nitrogen 34.62/37.65 $p > 0.05$, hematocrit 32.62/35.30 $p < 0.05$, ionized calcium 1.13/1.28 $p < 0.05$) (Figure 3, Table1).

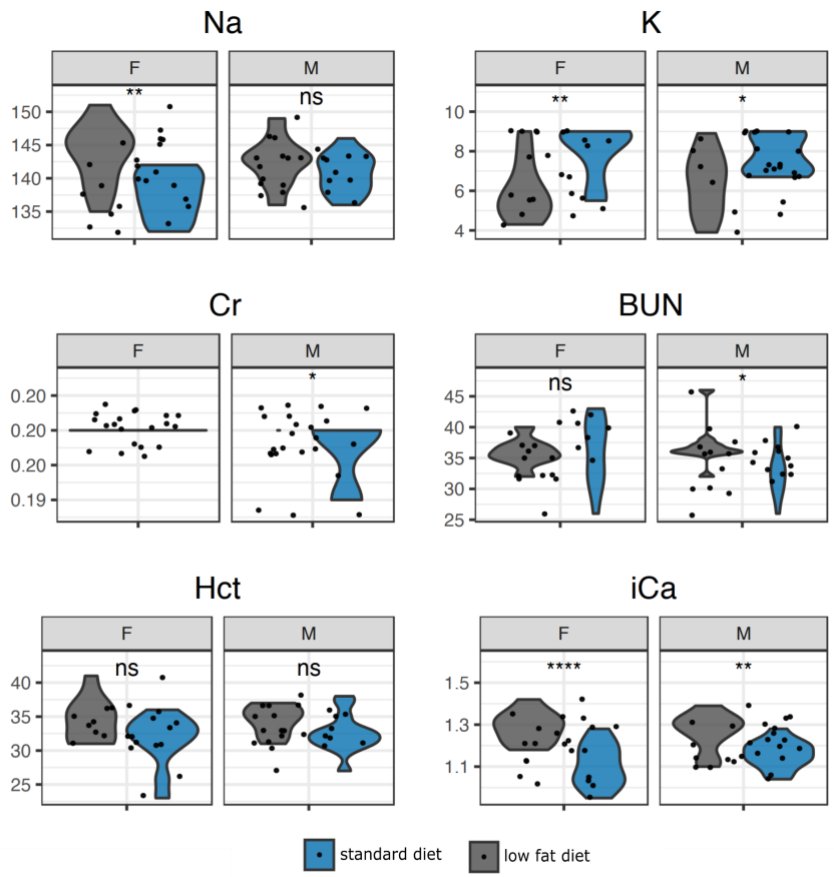

Figure 3

Violin plots showing the distribution of serum electrolyte measurements (Na = sodium, K = potassium, Crea = creatinine, BUN = blood urea nitrogen, Hct = hematocrit, iCa = ionized calcium) for female (F) and male (M) *Peromyscus eremicus*. Observations (n = 56, 28 each sex) are represented by black dots and violin colors denot diet: blue for standard diet and gray for low fat diet. Group means that are statically different are denoted with one or more * (student's t test, $p < 0.05$) and insignificant comparisons are denoted with ns (student's t test, $p > 0.05$). Note, the Abaxis i-STAT® Alinity analyzer does not measure creatinine below 0.2 mg/dL, and therefore the range for normal creatinine is truncated at this value.

Discussion

In light of current rapid climate change (IPCC, 2019), understanding how animals respond to environmental change is of paramount importance. The ability to adapt in place to changing climate will depend on a combination of adaptive plasticity and genetic variation, as well as the intensity of selection and length of generation times. Species unable to adapt in place could relocate but given the magnitude and rate of climate change and extent of habitat fragmentation, species may be unable to shift ranges (Beever et al., 2010; Henry et al., 2012; Sgrò et al., 2011). Extreme environments (e.g. high altitude, deep sea, deserts) and the organisms living in them represent natural experimental models, as the inhabitants have evolved behaviorally, physiologically, and genomically to persist in these challenging habitats. Understanding the mechanisms of survival under extreme conditions can provide insight into the potential for other species to respond to increasingly erratic and extreme climatic patterns. However, anthropogenic climate change is outpacing the evolutionary timescale on which many of these adaptive mechanisms evolved. Metabolic responses could aid in anticipating survival in novel scenarios but may not be sufficient for long term survival.

Water balance is complex and is in part modulated by diet. Different macronutrients yield different volumes of metabolic water via catabolism (carbohydrates = 0.6g metabolic water per gram, protein = 0.4g per gram, and fats = 1.07g per gram, Davidson et al., 1986). 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).

Moisture content can influence diet selection in wild desert mammals, which depend on preformed water in their diet (Frank, 1988; Orr et al., 2015). Because all food was dehydrated to match desert chamber conditions and by providing water *ad libitum,* we can clearly analyze the role that dietary fat plays in water balance in *Peromyscus eremicus.*

Here we expand on the long history of organismal water management studies in desert taxa (Bradford, 1974; MacMillen and Hinds, 1983; Frank, 1988; Hayes et al., 1998; Cortés et al., 2000; Albright et al., 2017; MacManes, 2017) to assess the contribution of diet to physiological adaptation to a dry environment. Like other desert mammals, *P. eremicus* rely on evaporative cooling for thermoregulation and thus, is expected to employ a frugal water economy when water intake is limited. We demonstrate diet-specific differences in the rate of evaporative water loss (RWL) and serum electrolyte values, and a consistent pattern of daily *de novo* lipogenesis (DNL, Colella et al., 2021b) that is not limited by dietary composition. These results support the hypothesis that water conservation is at least partially tied to diet composition and provide a deeper understanding of how desert animals maintain water homeostasis in hot, arid environments. Thus, we predict that a mismatch in physiological requirements, the availability of food type, and environmental conditions could significantly impact *P. eremicus* survival, and perhaps other desert-dwelling animals.

Rate of Water Loss

In our study, animals fed a low-fat diet lost more water than did animals fed a diet higher in fat (Figure 1c). Additionally, RWL was correlated with sex, with males losing more

water than females, mostly during their inactive daytime phase, when temperatures are elevated and relative humidity is lower, and during environmental transition phases when consuming the LFD. While the effects of temperature (Conley, 1985; Gordon, 1990; MacMillen and Hinds, 1983; Walsberg, 2000; Zhu et al., 2008) and weight (MacMillen and Hinds, 1983; Van Sant et al., 2012) on RWL in mammals are well described, with smaller-bodied mammals losing more water through evaporation and water loss increasing rapidly at high temperatures, less understood is how diet affects the RWL. Previous studies hypothesized that sexual dimorphism in water loss can be explained by differences in body size, metabolism, and respiratory rate (Cryan and Wolf, 2003), however, here we found no statistical difference in weight, consistent with other investigations of this species (Colella et al., 2021b; Manning et al., 2006).

Breakdown of fats in the SD is expected to result in the formation of more water. Therefore, in the absence of any source of water loss, the SD should lead to an increase in hydration compared to the LFD. Dehydration would occur if the amount of water lost is greater than the cumulative amount of water generated and consumed. Numerous studies have identified reduced respiratory water loss as a major physiological adaptation to maintaining positive water balance (Hart, 1971; MacMillen, 1972; Schmidt-Nielsen and Schmidt-Nielsen, 1951); however, not all diets have the same energy uptake (Withers, 1982). Diets with lower potential for metabolic water production, such as the LFD diet used in this experiment, would challenge survival because total evaporative water loss alone could result in water imbalance, specifically in a negative water balance that could not be compensated for by an increase in exogenous water consumption. Not much is known about *P. eremicus* foraging

strategies and whether foraging varies between sexes. We predict that if *P. eremicus* are forced to consume a LFD, they become more dehydrated, which likely has consequences for survival and ultimately, fitness. In hot, arid deserts, where extrinsic free water is severely limited, individual diet variation could significantly impact survival, particularly when diet is constrained.

Energy Expenditure

While studies have shown that diet composition influences EE (Acheson, 1990; Westerterp, 2004; Secor, 2009; Beale et al., 2018), there were no significant differences in EE found in our study. Factors that influence EE can be intrinsic (i.e., body mass, evolutionary history, reproductive stage, activity, or energy uptake) or extrinsic (i.e., habitat, climate, or social environment) (Speakman, 1997) and in wild populations, dietary switches tend to coincide with seasonal changes that affect resource quantity and/or quality (Noble et al., 2019). In the current study, we were able to isolate diet from other extrinsic and intrinsic factors to show that diet is likely not responsible for differences in EE. Consistent with other studies, we recorded a higher EE during the dark phase of the 24-hour cycle (Colella et al., 2021b) further supporting that metabolic rate is complex and may be modulated by the environment, activity, or other extrinsic/intrinsic factors.

Respiratory Quotient

Our results suggest that *de novo* lipogenesis (DNL), as indicated by an RQ > 1.0 during the light phase, is not limited by fat intake in this species. RQ, the ratio of O_2 consumed

to CO_2 produced, can inform rates of differential fuel consumption. 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) (Abreu-Vieira et al., 2015; Benedict, 1937; Levin et al., 2017) or during aerobic exercise (Whipp, 2007; Zagatto et al., 2012). In the experiment described here and in previous experiments on *P. eremicus* (Colella et al., 2021b), RQ is only greater than 1.0 during the light phase when animals are inactive, supporting DNL. While carbohydrates, fats, or protein are all burned as fuel, if present in excess, adipose fat can be easily stored in animals (Barboza et al., 2009). One mechanism for converting non-fat energy into a form that can be stored is DNL. The conversion of glucose to fats prior to entry into the citric acid cycle increases heat 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. Previous studies have shown that DNL can occur under high-carbohydrate and high-fat diets (Strable and Ntambi, 2010). This is supported in our study 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.

Electrolytes

To further understand the impacts of diet on physiological function of endotherms in desert environments, we collected serum electrolyte data from males and females fed diets with varying levels of fat. We established diet-specific differences for serum

sodium (Na), potassium (K), creatinine (Cr), blood urea nitrogen (BUN), hematocrit (Hct), and ionized calcium (iCa) values for *P. eremicus*. Electrolytes provide information on the overall metabolic state, renal function, body water content, and other core physiological functions (Kutscher, 1968), including hydration status (Cheuvront et al., 2010) or disease state. While some electrolytes were elevated in response to the experimental manipulation, others were not. Notably, the synthetic markers of pathological renal impairment, BUN and Creatinine, were not significantly different in any treatment group. 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 physiological stress in the form of elevated serum electrolytes in the absence of exogenous water (Boumansour et al., 2021; Kordonowy et al., 2017), though without apparent renal impairment.

Interestingly, we found several significant differences related to diet, several of which were sexually dimorphic. Sodium was found to be significantly elevated in females but not males (females SD: 140.50, females LFD: 143.60, $p = 0.004$, males SD: 140.71, males LFD: 137.50, $p = 0.210$) with *ad lib* access to water but fed a low-fat diet. This is particularly interesting as both males and females fed a low-fat diet had higher rates of water loss than when fed the standard diet. Elevated serum sodium in the context of normal renal function is a classic sign of dehydration, but significant dehydration does not appear to be the case here, as mice were given free access to water, did not lose weight, and there were no differences in hematocrit for any treatment group. Instead, dietary fats may themselves be responsible for changes in solute

balance. For instance, in rats, but not humans, differences in dietary fat were linked to changes in serum sodium (Friedman et al., 2012), but in the opposite direction to what we found (Abdel-Rahman, 2010). These changes were proposed to be aldosterone-mediated (Song et al., 2004), a process for which we have no data. Whatever 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.

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 the nature of the diet being critically important, particularly to the former. Examining the physiological responses of animals fed experimentally modified diets aids in our understanding of how animals may respond to changing environments where food availability varies. This will ultimately be important in predicting an animal's response to climate change because diet impacts water balance. To begin 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, a standard diet or a low-fat diet. Here, we present physiological measurements that show dietary fat is important for water regulation in the desert-adapted cactus mouse.

We found that *P. eremicus* had a variety of responses to a decrease in dietary fat 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 physiological stress. 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 both body temperature and activity monitoring can 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 regards 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.; 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 available also 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

Number of samples 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
T1	standard	233	256
	low fat	259	235
T2	standard	87	87
	low fat	87	87

GAMM tables

H2Omg ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(EE) + s(RQ)

	Estimate Std.	Error	t value	Pr(>|t|)	
intercept	1.372	0.129	10.625	< 2e-16	***
diet	0.642	0.149	4.304	1.70E-05	***
sex	0.448	0.141	3.007	0.003	**

	edf	Ref.df	F	p-value	
s(days)	1.992	1.992	170.560	<2e-16	***
s(diurnal)	7.949	8.000	683.880	<2e-16	***
s(EE)	6.630	6.630	21.190	<2e-16	***
s(RQ)	8.413	8.413	38.190	<2e-16	***

log(EE) ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(RQ)

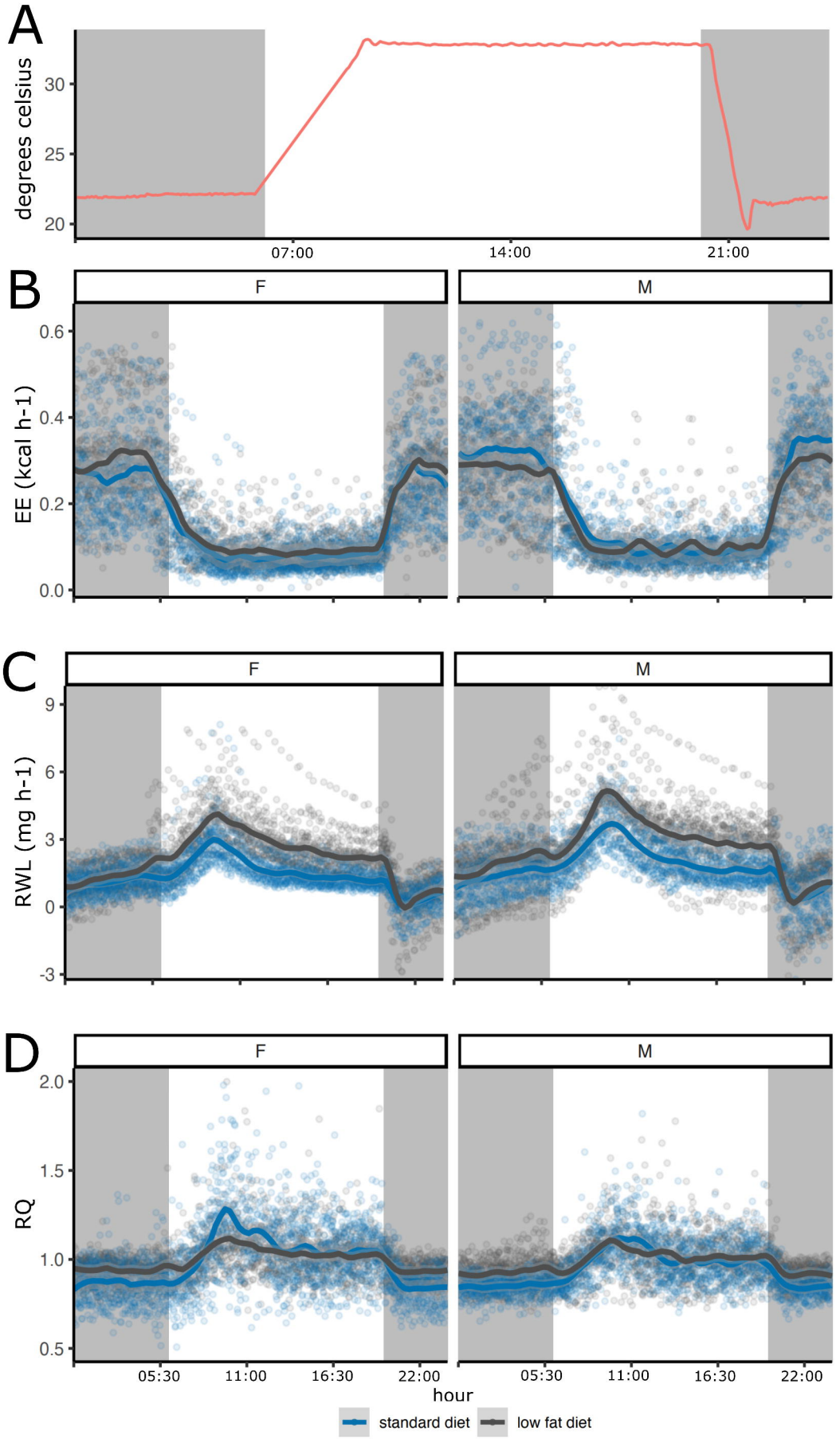
	Estimate Std.	Error	t value	Pr(>|t|)	
intercept	-2.017	0.063	-31.907	<2e-16	***
diet	0.042	0.073	0.578	0.563	
sex	0.098	0.073	1.341	0.180	

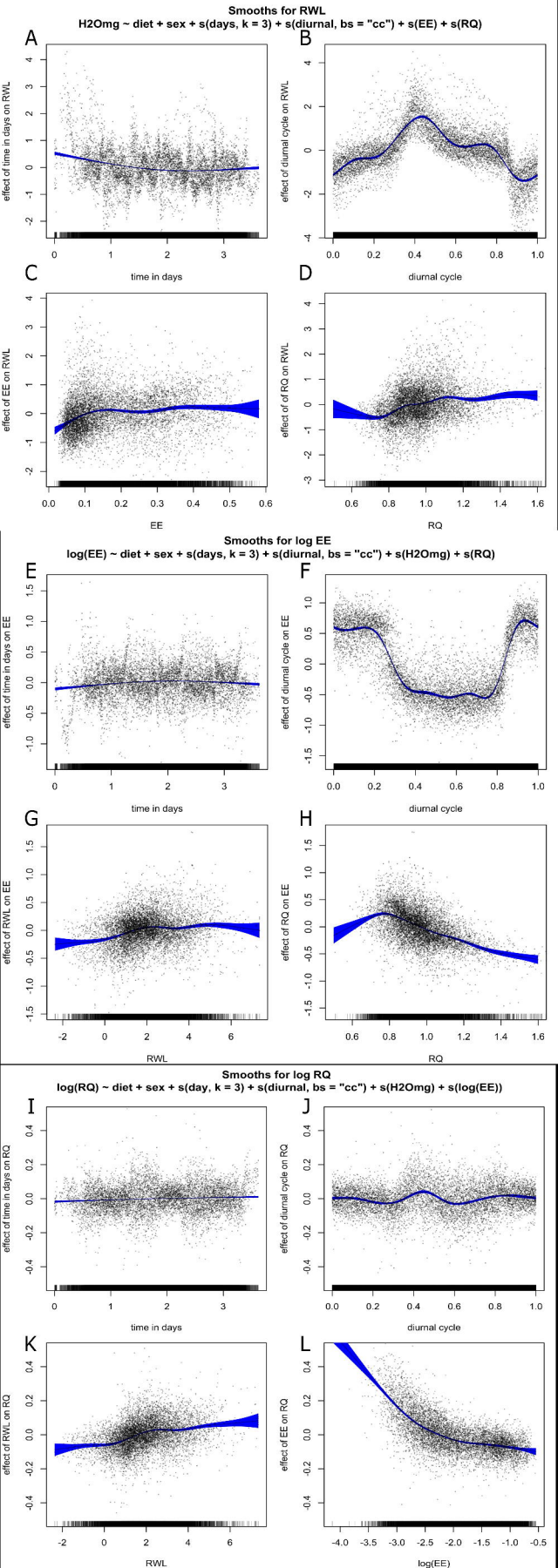
	edf	Ref.df	F	p-value	
s(days)	1.979	1.979	38.860	<2e-16	***
s(diurnal)	7.977	8.000	1482.280	<2e-16	***
s(H2Omg)	7.063	7.063	28.730	<2e-16	***
s(RQ)	8.154	8.154	180.690	<2e-16	***

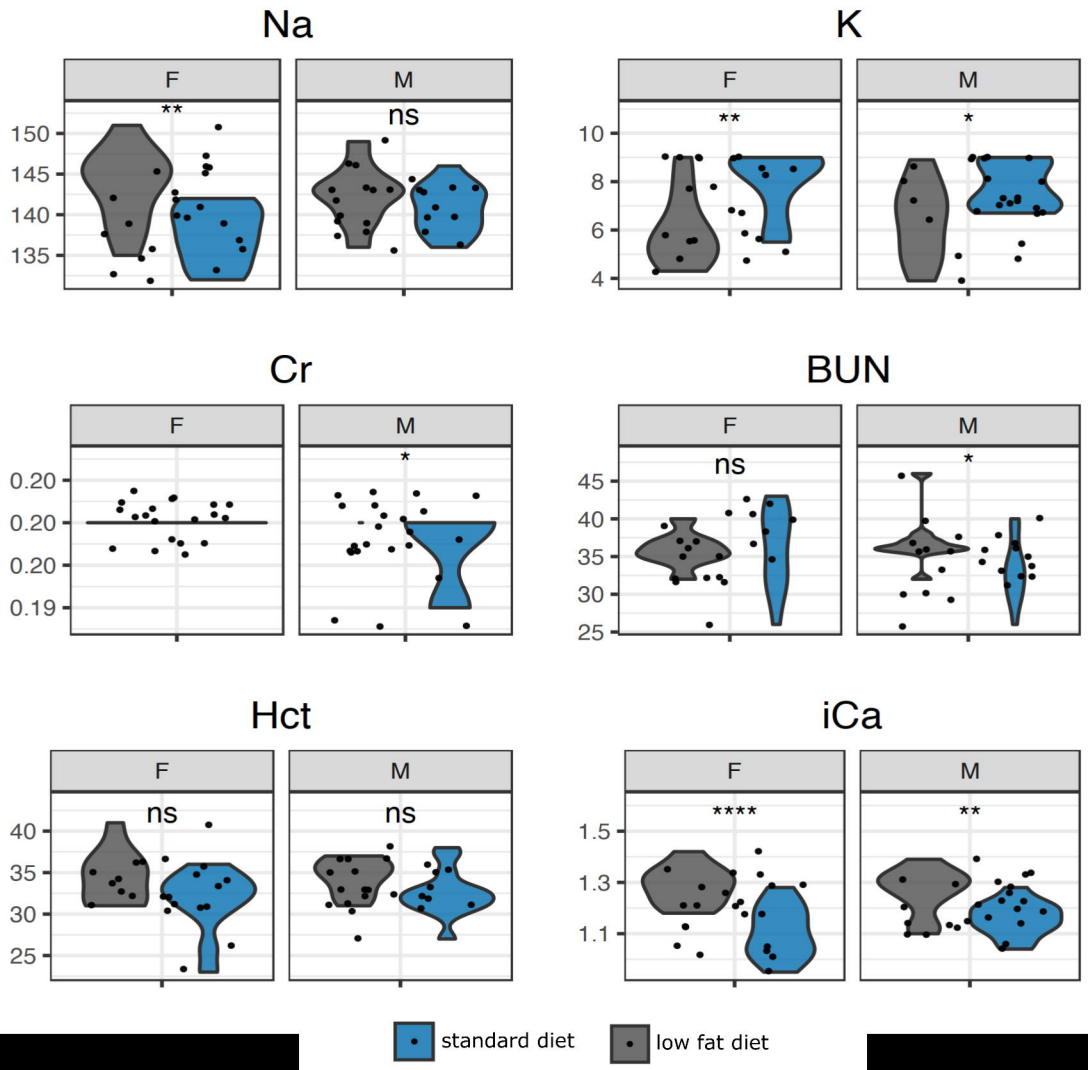
log(RQ) ~ diet + sex + s(day, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(log(EE))

	Estimate Std.	Error	t value	Pr(>|t|)
intercept	-0.049	0.034	-1.446	0.148
diet	0.025	0.039	0.640	0.522
sex	-0.008	0.039	-0.206	0.837

	edf	Ref.df	F	p-value	
s(days)	1.547	1.547	25.610	<2e-16	***
s(diurnal)	7.706	8.000	46.520	<2e-16	***
s(H2Omg)	7.387	7.387	48.360	<2e-16	***
s(log(EE))	7.149	7.149	359.650	<2e-16	***


A
degrees celsius
30
25
20
07:00
14:00
21:00
B
F
M
EE (kcal h-1)
0.6
0.4
0.2
0.0
C
F
M
RWL (mg h-1)
9
6
3
0
-3
D
F
M
RQ
2.0
1.5
1.0
0.5
05:30
11:00
16:30
22:00
hour
standard diet
low fat diet


Smooths for RWL
H2Omg ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(EE) + s(RQ)
A
effect of time in days on RWL
time in days
B
effect of diurnal cycle on RWL
diurnal cycle
C
effect of EE on RWL
EE
D
effect of of RQ on RWL
RQ
Smooths for log EE
log(EE) ~ diet + sex + s(days, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(RQ)
E
effect of time in days on EE
time in days
F
effect of diurnal cycle on EE
diurnal cycle
G
effect of RWL on EE
RWL
H
effect of RQ on EE
RQ
Smooths for log RQ
log(RQ) ~ diet + sex + s(day, k = 3) + s(diurnal, bs = "cc") + s(H2Omg) + s(log(EE))
I
effect of time in days on RQ
time in days
J
effect of diurnal cycle on RQ
diurnal cycle
K
effect of RWL on RQ
RWL
L
effect of EE on RQ
log(EE)


Na
F
M
**
ns
150
145
140
135
K
F
M
**
*
10
8
6
4
Cr
F
M
*
0.20
0.20
0.20
0.19
BUN
F
M
ns
*
45
40
35
30
25
Hct
F
M
ns
ns
40
35
30
25
iCa
F
M
****
**
1.5
1.3
1.1
standard diet
low fat diet