Glucocorticoids, Aerobic Physiology, and Locomotor Behavior in California Mice*

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Accepted 7/25/2012; Electronically Published 9/17/2012

Online enhancement: appendix table.

ABSTRACT

The glucocorticoid hormones corticosterone (CORT) and cortisol influence numerous physiological, morphological, and behavioral functions. However, few studies have addressed possible relationships between individual differences in glucocorticoid concentrations and whole-animal performance or metabolism. Because CORT is important in glucose regulation and energy metabolism and can influence activity levels, we hypothesized that individual variation in baseline circulating CORT levels would correlate with individual differences in energy expenditure (routine and maximal), aerobic physiology, voluntary exercise on wheels, and organ masses. We tested this hypothesis in the California mouse (*Peromyscus californicus*). We collected data from 54 adult, colony-bred mice on baseline CORT levels (measured near both the circadian peak and the circadian trough), voluntary wheel running and its energetic costs, maximal oxygen consumption during forced treadmill exercise ($\dot{V}O_{2\,(max)}$), basal metabolic rate, and relative organ masses. We found surprisingly few statistically significant relationships among CORT, energy metabolism, behavior, and organ masses, and these relationships appeared to differ between males and females. These findings suggest that individual differences in baseline CORT levels are not an important determinant of voluntary activity levels or aerobic performance in California mice.

* This paper was submitted in response to a call for papers for a Focused Issue on "Intraspecific Variation in Physiology and Behavior."

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Physiological and Biochemical Zoology 85(6):671–683. 2012. 1522-2152/2012/8506-1164$15.00. DOI: 10.1086/667809

Introduction

The glucocorticoids cortisol and corticosterone, steroid hormones secreted by the adrenal cortex, are important in glucose regulation and energy balance both under baseline conditions and in response to stress (Sapolsky et al. 2000; Romero and Butler 2007). Basal plasma glucocorticoid concentrations display a sinusoidal circadian rhythm, with the highest levels occurring just before the active period, consistent with their role in metabolism and energy partitioning (see Lightman et al. 2008 and Dickmeis 2009 for reviews; also Dallman et al. 1987). Circulating basal glucocorticoids are important for maintaining organismal homeostasis and in addition are thought to have permissive effects: at basal levels, glucocorticoids help the organism prepare for subsequent energetic challenges and potentially enhance the initial response to future stressors (Munck and Náray-Fejes-Tóth 1994; Sapolsky et al. 2000). The effects of glucocorticoids are time, dose, and context dependent (Munck and Náray-Fejes-Tóth 1992; Øverli et al. 2002; Mikics et al. 2007; Kralj-Fišer et al. 2010), pointing to the importance of distinguishing between basal (including both the nadir and the peak of the circadian rhythm) and poststress hormone levels.

Numerous studies have examined the relationships among glucocorticoid levels, metabolism, and whole-organism behavior and performance (Sinervo and Calsbeek 2003; Miles et al. 2007; Breuner et al. 2008; Campbell et al. 2009; Careau and Garland 2012). In general, adrenalectomy reduces activity levels, whereas administration of exogenous glucocorticoids has the opposite effect (Brown et al. 1982; Eaves et al. 1985; Wolkowitz 1994; Sandi et al. 1996). However, results are not always consistent among or within species. In addition, the above-listed studies tended to focus on extreme situations of either virtually no circulating glucocorticoids (adrenalectomy) or pharmacologically high levels of glucocorticoids (glucocorticoid administration). The few studies that have considered interactions among endogenous glucocorticoid levels, behavior, and locomotor physiology have generally found positive correlations between corticosterone (CORT) levels and physical or locomotor activity (mice and rats: Coleman et al. 1998; Girard and Garland 2002; Malisch et al. 2008; Stranahan et al. 2008). This relationship is expected, as locomotion is an energetically demanding and highly integrative behavior requiring the involvement of a number of physiological systems, including the endocrine system (Karasov 1992; Rising et al. 1994; Coleman et al. 1998; Koteja et al. 1999; Girard and Garland 2002; Feder et al. 2010), and would therefore be expected to be influenced by CORT (Wolkowitz 1994; Almon et al. 2008).

In two of the few studies highlighting relationships between baseline CORT levels and locomotion, Malisch et al. (2007, 2008)

showed that mice from four replicate lines selectively bred for high voluntary wheel running had elevated endogenous baseline CORT concentrations shortly before and shortly after the time of the circadian peak, compared to those from nonselected control lines; however, baseline CORT levels did not differ statistically between selected and control mice at the circadian nadir. The authors noted that increased baseline CORT concentrations may support increased locomotor activity, possibly through mobilization of energy reserves, and could be a correlated response to selection for increased voluntary exercise. These results suggest that a positive correlation would be expected between activity levels and baseline CORT concentrations, especially around the time of the circadian peak (i.e., high baseline CORT may be permissive of high activity; Sapolsky et al. 2000; Almon et al. 2008; Kalsbeek et al. 2012). The specific mechanisms by which glucocorticoids influence locomotion, however, are not well understood. CORT increases heart rate and vasodilation in skeletal muscle arterioles as well as gluconeogenesis and mobilization of liver glycogen and free fatty acids, all of which may contribute to metabolic processes important for activity (Munck and Náray-Fejes-Tóth 1994; Wingfield and Romero 2001; Almon et al. 2008; de Kloet et al. 2008).

The two studies by Malisch and colleagues found that selection on wheel-running behavior altered circulating CORT levels but did not investigate relationships between CORT and running behavior in individual mice. Interindividual variation in endocrine function is often overlooked in comparisons of group means, and it is unclear whether such individual variation contributes to functionally significant individual differences in regulation of physiological and behavioral systems (Zera and Harshman 2001; Piersma and Drent 2003; Guimont and Wynne-Edwards 2006; Williams 2008; Richardson et al. 2009; Careau and Garland 2012). In view of the critical role of the glucocorticoids in regulating metabolism, energy balance, and behavior and the fact that hormone secretion can vary among individuals within a species (Almon et al. 2008; Williams 2008), we were interested in possible relationships between individual differences in basal glucocorticoid concentrations and individual variation in whole-animal locomotor activity, performance, and metabolism.

We examined relationships among basal CORT (the major glucocorticoid in many rodent species), aerobic metabolism, and voluntary wheel running in the California mouse (*Peromyscus californicus*), a small, quadrupedal rodent. This species is unusual among mammals in having a monogamous mating system with extensive male parental care and minimal sexual size dimorphism (Gubernick and Alberts 1987; Gubernick 1988; Gubernick and Teferi 2000); consequently, some aspects of its endocrinology have been described. *Peromyscus californicus* has unusually high baseline CORT levels for a rodent (Glasper and DeVries 2005; Harris et al. 2011; Trainor et al. 2011; de Jong et al. 2012), as well as extremely dynamic diurnal CORT rhythms with very large changes in CORT concentration from trough to peak (Harris et al. 2012). As CORT is involved in mobilizing energy stores, this high baseline may be indicative of unusually high voluntary activity, with individual variation around the mean correlated with individual variation in exercise propensity or capacity. In that context, we were interested in the predictive value of baseline CORT for activity levels.

Therefore, we studied voluntary exercise in running wheels and concomitant energy expenditure, as well as basal and maximal aerobic metabolism, and tested whether these measures were related to CORT profiles. We collected three independent measures of activity (or metabolism) in an effort to evaluate individual variation. Voluntary wheel running is thought to be an ecologically relevant measure of normal, daily activity patterns in rodents (Dewsbury 1980; Chappell et al. 2004, 2007; Swallow et al. 2005), whereas basal and maximal measurements provide lower and upper limits, respectively, to rates of aerobic metabolism. We predicted that voluntary running performance, basal metabolic rate (BMR), and maximal oxygen consumption ($\dot{V}O_{2(max)}$) would be correlated with baseline CORT concentrations in *P. californicus*. Because these mice have large individual variation in baseline CORT levels (both throughout an individual's diurnal rhythm and among individuals), we predicted that if CORT plays an important role in mobilizing fuel for locomotion, then variation in baseline CORT would be correlated with metabolic and behavioral traits (particularly voluntary wheel running). In addition, we expected circadian peak baseline CORT values to be more variable and to have more predictive value, at least in terms of voluntary locomotor activity, than circadian nadir CORT values. Finally, we also examined organ masses, as glucocorticoids may have both direct and indirect effects on organ size (Dong et al. 2007; Shini et al. 2009). High CORT values may be negatively associated with masses of metabolically or immunologically active organs (e.g., heart, brain, spleen, musculoskeletal system), potentially to compensate for increased metabolic demands typically seen as a result of activation of the hypothalamic-pituitary-adrenal (HPA) axis (Elliott et al. 2003; Shini et al. 2009; Vahdatpour et al. 2009). Moreover, organ sizes (particularly those of metabolically active organs) may correlate with the upper and lower limits of aerobic metabolism (Konarzewski and Diamond 1995; Javed et al. 2010; Kolb et al. 2010; Ksiazek and Konarzewski 2012) and voluntary exercise (Chappell et al. 2007). For example, $\dot{V}O_{2(max)}$ is generally thought to be a good indicator of cardiopulmonary function and overall capacity for sustained energy expenditure, and therefore heart mass and lung mass are expected to be positively correlated with $\dot{V}O_{2(max)}$ (Weibel et al. 1991; Bishop 1999; Rezende et al. 2006). Given the role of CORT in energy metabolism, such organ masses might also be expected to correlate with CORT levels as evidence of a metabolic alteration or compensatory mechanisms resulting from high circulating CORT concentrations (Sapolsky et al. 2000).

Methods

Animals

A total of 54 *Peromyscus californicus* (25 males and 29 females) were used. Mice were bred at either the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia; 8 males, 18 females) or our colony at the University of California, Riverside

(UCR). The Stock Center colony was founded from about 60 individuals collected in the Santa Monica Mountains in California between 1979 and 1987; the UCR colony is derived from animals purchased from the Stock Center. All animals used in this study were housed in the UCR vivarium, as described previously (de Jong et al. 2009); mice from the Stock Center were housed at UCR for 215 ± 15 d (mean ± SEM; range: 107–385 days) before their use in this experiment. Mice were housed in 44 × 24 × 20-cm (length × width × height) cages with ad lib. access to food (Purina rodent chow, #5001) and water (see "Basal Metabolic Rate" for housing changes). Mice were housed under a 14L : 10D cycle, with lights on at 0500 hours; temperature was kept at approximately 23°C, with approximately 65% humidity. *Peromyscus californicus* were reproductively mature (no females were pregnant) and averaged 324 ± 21 d of age (range: 73–617 days) at the onset of metabolic testing. Body mass before BMR measurements was 38.0 ± 1.4 g (mean ± SEM) for males ($N = 25$) and 41.3 ± 1.9 g for females ($N = 29$).

Animals were housed in one of two social conditions, isolated or paired. Paired animals remained with one cagemate, either their own offspring or an adult conspecific of either sex, until metabolic testing. Isolated animals were separated from cagemates for at least 1 wk before basal blood samples were taken, to allow CORT levels to stabilize, as housing condition has been shown to influence CORT concentrations in this species (Glasper and DeVries 2005). Some animals in this study had been used previously in behavioral experiments.

Each mouse underwent collection of two initial blood samples, followed by a minimum of 5 d of rest before metabolic measurements, which occurred in the following order: voluntary activity (days 1–3), maximal oxygen consumption (day 3), and BMR (day 4). After BMR measurements, mice were euthanized, and organ masses were determined after dissection. The UCR Institutional Animal Care and Use Committee approved all procedures. UCR is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Blood Sampling

Each mouse was sampled at two different times, 1100 hours ($CORT_{AM}$) and 1800 hours ($CORT_{PM}$), in order to capture values near the circadian nadir and peak corticosterone values, respectively. Previously collected data (Harris et al. 2012) show that peak CORT concentrations (on this light schedule) occur at approximately 2000 hours and that nadir CORT concentrations occur between 0400 and 0800 hours. CORT values begin to rise at 1200–1600 hours. Therefore, $CORT_{AM}$ should be close to nadir, and $CORT_{PM}$ values are expected to be near peak levels. The order of blood sampling (peak vs. nadir) was randomized among mice.

Mice were anesthetized with isoflurane gas, and approximately 70 μL of blood was collected into a heparinized microhematocrit capillary tube via the retro-orbital sinus within 4 min (average time ± SEM: 94 ± 4 s) after the cage was disturbed. Blood was centrifuged for 12 min at 13,300 rpm and 4°C. Hematocrit was measured, and plasma was stored at −80°C until assay. The $CORT_{AM}$ and $CORT_{PM}$ samples from each mouse were collected at least 2 d apart to minimize hematocrit changes due to blood loss; the two samples were taken from alternate eyes. Mean ± SEM hematocrit values from the first and second blood samples were 46.3% ± 0.35% and 45.3% ± 0.45%, respectively ($r = 0.503$, $t = 2.790$, $P = 0.008$). Animals were allowed to recover for at least 5 d from the time of the second blood sample until the beginning of the metabolic-testing phase to allow hematocrit values to return to normal.

Corticosterone Assay

Plasma samples were assayed for CORT with a commercially available kit (double-antibody corticosterone radioimmunoassay, 07–120102, MP Biomedicals, Costa Mesa, CA) that had been fully validated for use with California mouse plasma (Chauke et al. 2011). Plasma samples were diluted either 1 : 400 or 1 : 800, depending on the expected concentration of hormone in the sample (higher values were expected at the 1800-hours time point); samples were assayed in duplicate. Intra- and interassay coefficients of variation were 1.40% and 6.96%, respectively. One mouse with CORT data available from only one of the two blood samples was excluded from analyses, and one additional mouse was excluded because its $CORT_{AM}$ values were higher than its $CORT_{PM}$ values, indicating an aberrant circadian rhythm. While we have not explicitly tested CORT repeatability in *P. californicus*, previous data show consistent circadian rhythmicity in CORT levels (Harris et al. 2012). Specifically, within our colony of mice, pooled data from two previous studies (Chauke et al. 2011; Harris et al. 2011) indicate that $CORT_{AM}$ values are repeatable within individuals ($r = 0.356$, $P = 0.036$, $N = 35$). In addition, studies on other species have demonstrated that individual CORT measures are generally repeatable (Wada et al. 2008; Cockrem et al. 2009; Angelier et al. 2010). No apparent baseline CORT differences between Stock Center animals and UCR-bred animals have been noted in previous studies (see Chauke et al. 2011 and Harris et al. 2011 for CORT values of Stock Center and UCR-bred mice, respectively).

Voluntary Exercise on Wheels

Plexiglass-enclosed running wheels allowed concurrent measurement of voluntary wheel-running behavior and gas exchange (Chappell et al. 2004, 2007; Rezende et al. 2006, 2009; Dlugosz et al. 2009). The enclosures contained a stainless steel running wheel (circumference: 1.12 m; effective volume: 17 L) attached to a standard housing cage containing ad lib. food (standard rodent chow), water, and a fan that circulated air throughout the chamber. Dry air was supplied at 2,500 mL/min, controlled by Tylan (Billerica, MA) or Sierra (Monterey, CA) mass flow controllers. Wheel chambers were held in an environmental cabinet maintaining temperature at 27.0° ± 0.4°C. An oxygen analyzer (Oxzilla; Sable Systems, Henderson,

NV) subsampled excurrent air (dried with magnesium perchlorate) at 100 mL/min. Three-minute reference readings were taken every 42 min. Data were sampled every 1.5 s and recorded in Warthog LabHelper software (http://www.warthog.ucr.edu).

Measurements of Voluntary wheel-running lasted approximately 47.5 h for each mouse. LabAnalyst software (http://www.warthog.ucr.edu) was used to correct baselines, account for lag time, interpolate reference readings, and calculate rates of gas exchange (see Chappell et al. 2004).

To minimize effects of electrical noise and account for system response times, metabolic data were smoothed and instantaneous corrections (Bartholomew et al. 1981) were used. To avoid long lag times (because of large scrubber volume) or frequent CO_2 scrubber changes, CO_2 was not removed before oxygen measurements. A constant respiratory quotient (RQ = 0.85) was assumed, and oxygen consumption ($\dot{V}O_2$) was calculated as

$$\dot{V}O_2 = \frac{\dot{V}(F_iO_2 - F_eO_2)}{1 - F_eO_2(1 - RQ)}, \quad (1)$$

where $\dot{V}$ is the flow rate and F_iO_2 and F_eO_2 are the fractional incurrent and excurrent oxygen concentrations, respectively.

Slopes (incremental cost of transport, iCOT) and intercepts of the speed-versus-$\dot{V}O_2$ regression for each individual were calculated, and data were obtained with the LabAnalyst stepped sampling procedure (1-min means separated by 3 min), in an effort to reduce autocorrelation and obtain $\dot{V}O_2$ values statistically independent of each other (detailed in Chappell et al. 2004). Speeds less than 0.5 m min^{-1} were removed to eliminate effects of tachometer electrical noise. Variables measured for wheel metabolic trials are displayed in table 1.

Maximal Metabolic Rate

Immediately after voluntary activity measurements, maximal rate of oxygen consumption ($\dot{V}O_{2(max)}$) in forced exercise was measured, either in a small, enclosed treadmill (Rezende et al. 2006) or in a small, enclosed wheel (circumference: 51.8 cm; effective volume: 900 mL; Chappell and Dlugosz 2009). Results from the two methods did not differ statistically in a comparison of nine animals measured on the treadmill and 38 measured in the wheel system (two-tailed $P = 0.626$). Flow rates (2,000 sccm [standard cubic cm/min] in the treadmill, 2,400 mL/min in the wheel) and gas concentrations were recorded every second with LabHelper software, with references taken at the beginning and end of each trial. Excurrent air was subsampled at about 150 mL/min and dried with magnesium perchlorate. Dried air flowed through a CO_2 analyzer (LiCor LI-6251), was scrubbed of CO_2 and redried (soda lime and Drierite), and then passed through an O_2 analyzer (Applied Electrochemistry S-3A). Oxygen consumption was calculated as

$$\dot{V}O_2 = \frac{\dot{V}(F_iO_2 - F_eO_2)}{(1 - F_eO_2)}. \quad (2)$$

As with the voluntary running wheel, instantaneous corrections (Bartholomew et al. 1981) were used to accurately record rapid changes in metabolism.

Mice were given several minutes to acclimate to the wheel or treadmill. When the test began, speed was initially low. Most mice oriented quickly and ran well. We matched the speed of the treadmill to the behavior of the animals. Speed was increased approximately every 30 s until $\dot{V}O_2$ did not increase or until the mouse refused to continue running. After the trial ended, mice were allowed to recover before being removed.

Basal Metabolic Rate

Several hours after the $\dot{V}O_{2(max)}$ trial, mice were fasted (with access to water) for 10–12 h. BMR measurements were taken over an 8-h period during the animals' inactive period (0800–1600 hours). Measurements were made in Plexiglass metabolic chambers (volume: 525 mL) with small amounts of bedding but without food or water. Air flow rates were approximately 600 mL min^{-1}. Metabolic chambers were kept in an environmental chamber, and temperature was maintained at 28°–30°C. Subsampled excurrent air was dried and passed through an Applied Electrochemistry oxygen analyzer (Pittsburgh, PA). Temperature, flow rates, and gas concentrations were recorded every 4 s, with 3-min references occurring every 42 min. The $\dot{V}O_2$ was computed with equation (2). The lowest continuous 10-min average $\dot{V}O_2$ represents the BMR.

Morphology

After metabolic measurements, mice were euthanized with CO_2 and dissected. We removed, fat-trimmed, rinsed, blotted, and weighed the following organs: heart, lungs, liver, spleen, stomach, cecum, large intestine, small intestine, reproductive organs (whole reproductive tract in females; testes plus epididymi in males), brain, and the remaining musculoskeletal system. Stomach, cecum, and small and large intestines were emptied before weighing, and their masses were added together to yield "gut" mass. Organs were dried at 55°C and reweighed until they reached constant mass; dry masses were used in analyses.

Statistics

For a few individuals, we did not obtain all measurements, so some animals were excluded from various analyses. Metabolic and body mass data were $\log_{10}$ transformed before analyses to improve normality (body mass) or linearity of relationships with body mass and/or the normality of residuals from allometric regressions. Because organ size, locomotor abilities, and metabolic physiology are strongly influenced by body mass, we used body mass residuals in comparisons. Residuals were computed from regressions on body mass (and age, where appropriate). Residuals and correlations were calculated separately for males and females. For males, the following variables were analyzed using body mass– and age-corrected residuals: gut mass, kidney mass, musculoskeletal mass, and $\dot{V}O_{2(1\,min)}$. For females, body

Table 1: Metabolic and behavioral variables measured and measures of circulating corticosterone levels

Symbol	Description	Units
DEE	Daily energy expenditure ($\dot{V}O_2$ averaged over 24-h recording period)	mL O_2 d^{-1}
RMR	Resting metabolic rate (minimum $\dot{V}O_2$ averaged over 10 min), measured during voluntary tests	mL O_2 d^{-1}
Distance run	Total distance run over 24-h recording period	m d^{-1}
Run time	Total time spent running over 24-h recording period	min d^{-1}
$\dot{V}O_{2\,(1\,min)}$	Maximum voluntary $\dot{V}O_2$ averaged over 1 min	mL O_2 min^{-1}
V_{max}	Maximum voluntary running speed averaged over 1.5-s sample interval	m min^{-1}
$V_{max\,(1)}$	Maximum voluntary speed averaged over 1 min	m min^{-1}
iCOT	Incremental cost of transport (slope of speed vs. $\dot{V}O_2$ regression using 1-min means separated by 3 min)	mL O_2 m^{-1}
Intercept	Intercept of speed versus $\dot{V}O_2$ regression	mL O_2 min^{-1}
Postural cost	Intercept − RMR (cost associated with maintaining an upright position during locomotion)	mL O_2 min^{-1}
N_{Bout}	Number of independent running bouts over 24-h recording period	
D_{Bout}	Mean duration of running bouts	min
Max_{Bout}	Maximum duration of running bouts	min
$\dot{V}O_{2\,(max)}$	Maximal $\dot{V}O_2$ over 1 min, measured during forced exercise	mL O_2 min^{-1}
BMR	Basal metabolic rate (lowest continuous $\dot{V}O_2$ over 10 min)	mL O_2 min^{-1}
$CORT_{AM}$	Total corticosterone measured at 1100 hours	ng/mL
$CORT_{PM}$	Total corticosterone measured at 1800 hours	ng/mL
ΔCORT	$CORT_{PM}$ − $CORT_{AM}$	ng/mL

mass– and age-corrected residuals were used for distance, run time, $V_{max\,(1)}$, D_{Bout}, Max_{Bout}, BMR, gut mass, kidney mass, and musculoskeletal mass (see table 1 for definitions of variables). CORT concentrations were untransformed (Romero 2004). All data are presented in untransformed units. The significance level α was 0.05 (two-tailed). To control for false discovery rates (FDRs) in multiple simultaneous tests (Benjamini and Hochberg 1995), we used the q-value procedure ("Qvalue" library; R statistical package). Both raw and FDR-adjusted probabilities are presented. A post hoc power analysis (http://www.quantitativeskills.com/sisa/statistics/correl.htm) using separate male and female correlations (and sample sizes therein) was performed, and all correlations that were significant after FDR corrections indicated a level of power well above the generally accepted 0.8. Other analyses included regressions of $\dot{V}O_2$ on body mass and generalized-linear-model procedures (used to compute mass and/or age residuals) in SPSS, version 11.0 for Mac OS X.

Results

Voluntary Wheel Running

As with other rodent species that have been tested (Chappell et al. 2004, 2007; Rezende et al. 2005, 2006, 2009; Dlugosz et al. 2009), California mice in this study exhibited substantial voluntary running in the enclosed-wheel metabolic chambers. Mean ± SEM daily distance run and time spent running were 2.88 ± 0.32 km and 147 ± 13 min, respectively, and there was considerable variation among individuals. The number of daily running bouts, running-bout durations, and running speeds varied greatly (table 2).

Many measures of voluntary activity and the associated metabolic rates were intrinsically or intuitively correlated (e.g., run time and run distance; daily run distance and daily energy expenditure [DEE]). After FDR adjustment, female DEE was significantly correlated with resting metabolic rate (RMR, minimum $\dot{V}O_2$ averaged over 10 min, measured during voluntary tests; $r = 0.609$, $P = 0.002$), voluntary $\dot{V}O_{2\,(1\,min)}$ (maximum $\dot{V}O_2$ averaged over 1 min; $r = 0.715$, $P < 0.001$), and intercept ($r = 0.860$, $P < 0.001$). In females only, the slope of the speed-versus-metabolic-rate regression (iCOT) showed significant negative correlations with maximum voluntary speed over 1.5 s (V_{max}; $r = -0.704$, $P < 0.001$), maximum voluntary speed sustained over 1 min ($V_{max\,(1)}$; $r = -0.661$, $P = 0.001$), and postural cost ($r = -0.635$, $P = 0.001$). After correction for body mass, postural cost (i.e., the cost associated with maintaining an upright body position during locomotion; see table 1) was significantly correlated with DEE in both sexes (males: $r = 0.730$, $P = 0.001$; females: $r = 0.616$, $P = 0.002$; see table A1 in the online edition of *Physiological and Biochemical Zoology*).

We found few correlations between voluntary wheel running and organ mass. For DEE in females, only dry heart mass and dry liver mass were significantly correlated after FDR adjustment ($r = 0.575$, $P = 0.004$ and $r = 0.608$, $P = 0.002$, respectively; table A1). In males, there were no significant correlations between wheel running and organ mass.

Corticosterone

We used three different measures of plasma CORT concentrations: $CORT_{AM}$, $CORT_{PM}$, and ΔCORT ($CORT_{PM} - CORT_{AM}$).

Table 2: Voluntary wheel-running measurements for male and female California mice

	Males ($N = 25$)		Females ($N = 28$)	
Trait	Mean (SD)	Range	Mean (SD)	Range
Distance run	2,857.6 (1,723.2)	531.8–6,697.7	2,900.6 (2,784.7)	140.0–11,700.4
Run time	154.95 (80.49)	20.91–307.83	139.24 (104.90)	20.90–459.15
N_{BOUT}	349.98 (191.59)	50.0–897.0	293.54 (138.99)	102.0–544.5
D_{BOUT}	26.65 (6.92)	15.91–39.45	27.07 (13.40)	11.19–64.19
V_{max}	55.45 (12.24)	38.98–84.26	55.57 (12.34)	18.89–78.48
$V_{max\,(1)}$	35.58 (10.70)	21.67–67.54	35.22 (10.73)	12.42–54.80
$\dot{V}O_{2\,(1\,min)}$	4.02 (1.00)	2.80–7.34	4.37 (.80)	2.44–6.59
DEE	1.60 (.25)	1.19–2.35	1.86 (.41)	.95–2.97
RMR	.80 (.17)	.54–1.29	.99 (.27)	.55–1.78

Note. See table 1 for explanation of abbreviations and units.

California mice have a large range of daily baseline CORT values (i.e.. a large ΔCORT) and very high circulating CORT concentrations, compared to those of most other rodents (Glasper and DeVries 2005; Chauke et al. 2011). Average $CORT_{PM}$ values, measured near the peak of the circadian cycle, are nearly tenfold higher than $CORT_{AM}$ values (close to nadir). As we expected, because $CORT_{AM}$ values are generally low and $CORT_{PM}$ values are much higher and more variable, the correlation between $CORT_{AM}$ and $CORT_{PM}$ ($r = 0.186$) is quite low. The notation ΔCORT was used to quantify individual variation in CORT measures for each mouse. Although the difference was not statistically significant, ΔCORT values tended to be higher in females (1,425 ± 135 ng/mL; range: 24–3,683 ng/mL) than in males (1,286 ± 137 ng/mL; range: 13–2,782 ng/mL), and females tended to have a slightly broader range in values (107–2,863 ng/mL) than males (276–2,692 ng/mL). In general, mice of either sex housed with a conspecific tended to have lower and less variable CORT concentrations than mice housed alone (fig. 1). However, neither $CORT_{AM}$, $CORT_{PM}$, nor ΔCORT in males or females differed significantly between housing conditions.

Given the broad range of physiological and behavioral functions modulated by glucocorticoid hormones, we found surprisingly few relationships between plasma CORT concentrations and metabolic, behavioral, or morphological measures. In both sexes, ΔCORT was significantly correlated with $CORT_{PM}$ but not with $CORT_{AM}$, possibly because $CORT_{PM}$ was highly variable, as compared to $CORT_{AM}$, or because of the sheer difference in magnitude between $CORT_{AM}$ and $CORT_{PM}$ values. After FDR adjustments, ΔCORT and $CORT_{PM}$ were negatively and significantly correlated with distance run in males, and there was a strong negative relationship between $\dot{V}O_{2\,(max)}$ and $CORT_{AM}$ in females (table 3).

Morphology

After FDR corrections, relatively few significant correlations were found among residual dry organ masses, and these tended to differ between males and females (table A1). In females, dry brain mass was negatively correlated with dry musculoskeletal mass ($r = -0.590$, $P = 0.004$), and dry liver mass was positively correlated with dry spleen mass ($r = 0.736$, $P < 0.001$). In males, dry gut mass and dry kidney mass were positively correlated ($r = 0.670$, $P < 0.001$).

Housing Condition, Activity, BMR, and $\dot{V}O_{2\,(max)}$

In males, housing condition was a significant predictor of BMR ($r = 0.573$, $P = 0.016$), indicating that male mice housed with a conspecific tended to have higher BMR than those housed alone. There were no statistically significant relationships between housing condition and metabolic or behavioral variables in females.

We found few significant correlations among BMR and performance measures. Maximal oxygen consumption measured during forced exercise ($\dot{V}O_{2\,(max)}$) was positively correlated with but, on average, well above maximum oxygen consumption measured during voluntary exercise ($r = 0.420$, $t = 11.771$, $P < 0.001$; fig. 2). Only 2 of 47 mice had voluntary $\dot{V}O_2$ values that exceeded forced $\dot{V}O_{2\,(max)}$ (5.8 vs. 3.5 mL O_2/min in one individual and 3.1 vs. 2.9 mL O_2/min in the other). In addition, before FDR corrections, $\dot{V}O_{2\,(max)}$ in males was positively correlated with BMR and mean hematocrit. After mass compensation and FDR correction, BMR was not significantly correlated with $\dot{V}O_{2\,(max)}$ or DEE in either sex. In females, BMR was positively correlated with dry brain mass and dry musculoskeletal mass before, but not after, FDR corrections (table A1).

Discussion

Our primary goal was to characterize individual variation in baseline CORT profiles in relation to voluntary wheel running, aerobic physiology, and morphology. Our measures of baseline circulating CORT concentrations are consistent with those previously reported in *Peromyscus californicus* (Chauke et al. 2011; Harris et al. 2011; Trainor et al. 2011; de Jong et al. 2012). Significant relationships of baseline CORT levels with voluntary activity and aerobic performance were few in number and differed between the sexes. In addition, we found few significant relationships among morphological measures, and these also

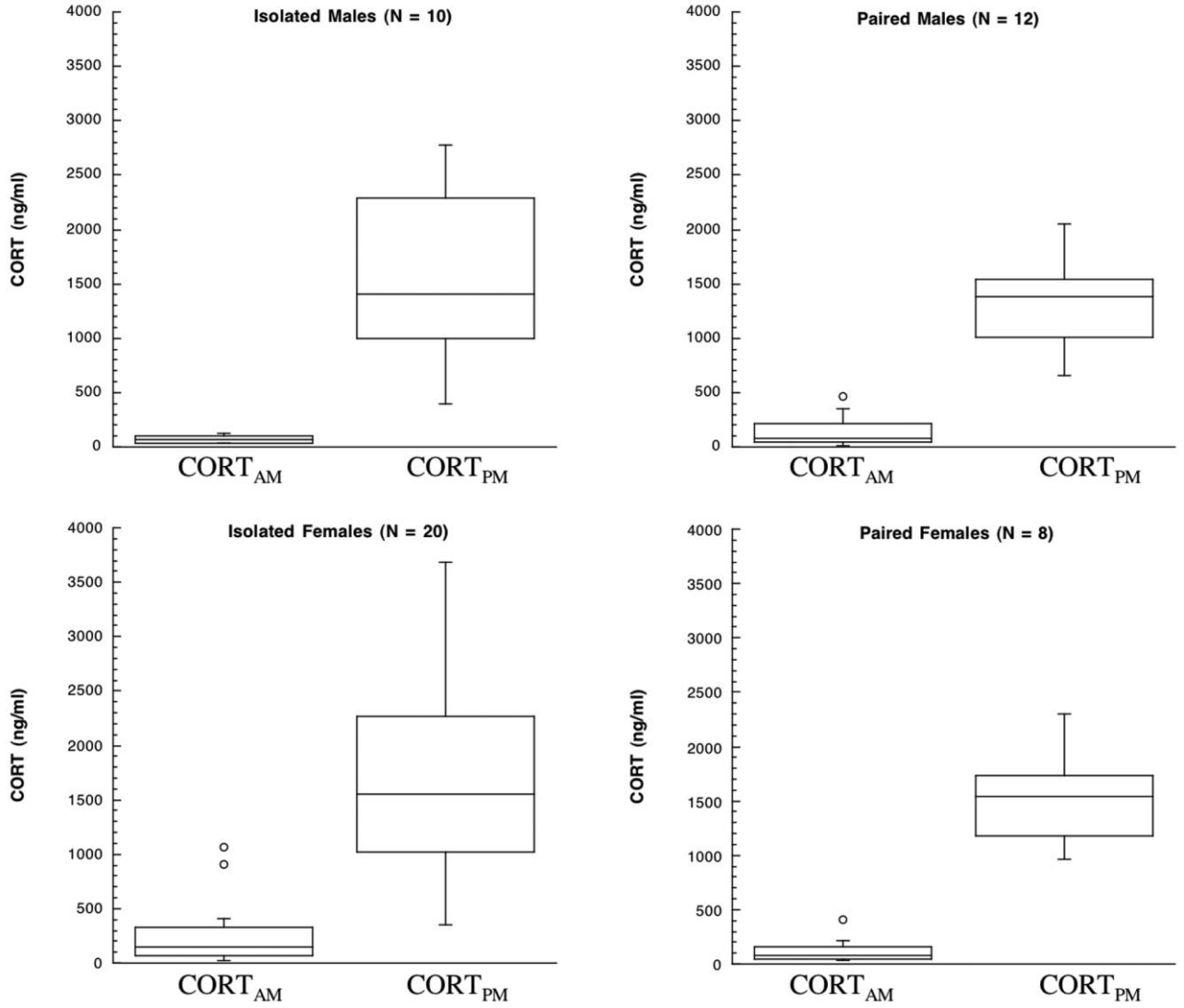

Figure 1. Baseline plasma corticosterone (CORT) concentrations around the times of the circadian nadir (1100 hours, $CORT_{AM}$) and the circadian peak (1800 hour, $CORT_{PM}$), in male and female California mice that were housed either individually (isolated) or in pairs. The bottom and top of each box plot represent the first and third quartiles, respectively. The horizontal line within each box represents the median. Whiskers indicate the range. Open circles are for outliers (more than 3 SD from the mean).

differed between males and females. Overall, these data suggest that individual differences in baseline CORT levels are not predictive of aerobic performance or voluntary activity levels in California mice.

Baseline CORT

Behavior, Performance, and Metabolism. Somewhat surprisingly, baseline CORT concentrations had little predictive value for voluntary running behavior or aerobic metabolism (table 3). Despite the relatively high baseline CORT levels in *P. californicus*, these mice show voluntary running performance (speed, running time, distance) similar to that of other rodents that have not been selectively bred for exercise capacity (e.g.. deer mice *Peromyscus maniculatus*: Chappell et al. 2004; Mongolian gerbils *Meriones unguiculatus*: Chappell et al. 2007; and several wild-caught rodents: M. A. Chappell, unpublished data). The measure ΔCORT ($CORT_{PM} - CORT_{AM}$; the circadian range in individual baseline CORT concentrations) was used to quantify individual variation in CORT measures for each mouse. Our study was designed so that blood samples were collected at consistent times, but it is not likely that we captured nadir or peak values for all individuals. If true nadir or peak values were missed or if timing of the diurnal rhythm was inconsistent among individuals, then we would expect fewer correlations between CORT and behavior or performance.

Our results suggest that large baseline ΔCORT levels have a primarily negative relationship with voluntary running levels

Table 3: Summary of Pearson correlations involving baseline plasma corticosterone (CORT) concentrations in California mice

	Males (N = 17–25)		Females (N = 22–29)	
Variables	r	P (two-tailed)	r	P (two-tailed)
ΔCORT; $CORT_{PM}$	.985	**<.001**	.945	**<.001**
$CORT_{AM}$; log $\dot{V}O_{2(max)}$	−.148	NS	−.648	**.001**
ΔCORT; distance run	−.719	**.001**	.201	NS
$CORT_{PM}$; distance run	−.706	**.002**	.179	NS
$CORT_{PM}$; run time	−.603	.010	.236	NS
ΔCORT; run time	−.637	.006	.265	NS
$CORT_{PM}$; D_{BOUT}	−.562	.019	−.033	NS
ΔCORT; D_{BOUT}	−.544	.024	.051	NS
$CORT_{AM}$; dry gut	.550	.007	−.192	NS
ΔCORT; dry kidney	.451	.035	−.051	NS

Note. All CORT correlations listed were significant ($\alpha = 0.05$) before false-discovery-rate (FDR) corrections; boldface indicates significance after FDR corrections (males: pi0 = 0.658, $\alpha = 0.002$; females: pi0 = 0.718, $\alpha = 0.004$). Residuals were taken with mass and age where necessary, and metabolic measures were $\log_{10}$ transformed. A total of 79 nonsignificant correlations with CORT are excluded from this table. See table 1 for explanation of abbreviations. NS = not significant.

in males but not in females. Male mice with a larger range in daily CORT values or with higher CORT concentrations at 1800 hours (high $CORT_{PM}$) do not voluntarily run as far and, although not significantly so, tend to engage in shorter running bouts and have shorter total voluntary running time than male mice with a smaller ΔCORT or lower $CORT_{PM}$ levels. These results may indicate that, contrary to our expectations, mice with increased circadian variation or plasticity in baseline CORT measures are less likely to mobilize energy stores for fueling locomotion. In line with this finding, we found that females with high $CORT_{AM}$ have lower $\dot{V}O_{2(max)}$ values than females with lower $CORT_{AM}$, again suggesting that high baseline CORT levels are detrimental to a female's performance (we found no significant relationship between $\dot{V}O_{2(max)}$ and CORT in males).

Many studies have reported correlations (albeit not always consistent in direction) between stress-induced glucocorticoid levels and metabolic rates in a variety of organisms, often in response to changing environmental conditions (Sapolsky et al. 2000; Vegiopoulos and Herzig 2007; Peckett et al. 2011). Because of the overwhelming support for glucocorticoid-mediated changes in whole-organism metabolism, it is reasonable to expect that baseline CORT may have substantial permissive effects on the behavior and physiology of an organism (Sapolsky et al. 2000; Almon et al. 2008; Kalsbeek et al. 2012). For this reason, the lack of strong associations between individual variation in baseline CORT concentrations and BMR or RMR is particularly interesting.

Housing. Aside from the potential effects of CORT on behavior and physiology, a number of factors could potentially influence variability in CORT itself. Within our data set, it is notable that housing condition was not a significant predictor of any CORT measure, indicating that the mean morning, evening, and ΔCORT values did not differ reliably between individually housed and pair-housed mice. California mice are highly social under natural conditions, typically living in monogamous pairs (Ribble and Salvioni 1990; Gubernick and Teferi 2000). Hence, solitary animals would be expected to be under stress. Despite the groups having statistically similar mean CORT values, individually housed male and female California mice tended to have a much larger range in ΔCORT values (~100 to ~2,900 ng/mL) than mice housed in pairs (~800 to ~2,200 ng/mL; fig. 1). The lack of statistical significance is surprising, because a previous study found that being housed with a conspecific greatly reduced CORT concentrations in male California mice, suggesting that isolation may chronically stimulate the HPA endocrine axis in this monogamous rodent (Glasper and DeVries 2005). One possible explanation for this disparity is that the pair-housed mice in our study lived in a variety of different social configurations, including pair-housing with one of their own offspring or with a same-sex or opposite-sex adult, which may have increased variability in basal CORT levels. We have previously found, however, that morning basal plasma CORT concentrations did not differ between male or female California mice housed with a same-sex pairmate and those with an opposite-sex pairmate (Chauke et al. 2011).

Activity, Metabolism, and Organ Mass

To more fully interpret individual differences in baseline CORT, we also investigated relationships among basal and maximal aerobic metabolism, organ morphology, and voluntary locomotor behavior. Values for BMR, $\dot{V}O_{2(max)}$, voluntary behavior, and organ masses in California mice in this study fall within ranges expected by comparison with other, similarly sized rodents (laboratory mice *Mus domesticus*: Swallow et al. 2005; Rezende et al. 2009; deer mice: Chappell et al. 2004; Russell and Chappell 2007; Mongolian gerbils: Chappell et al. 2007). Locomotion can be energetically expensive, and activity is likely

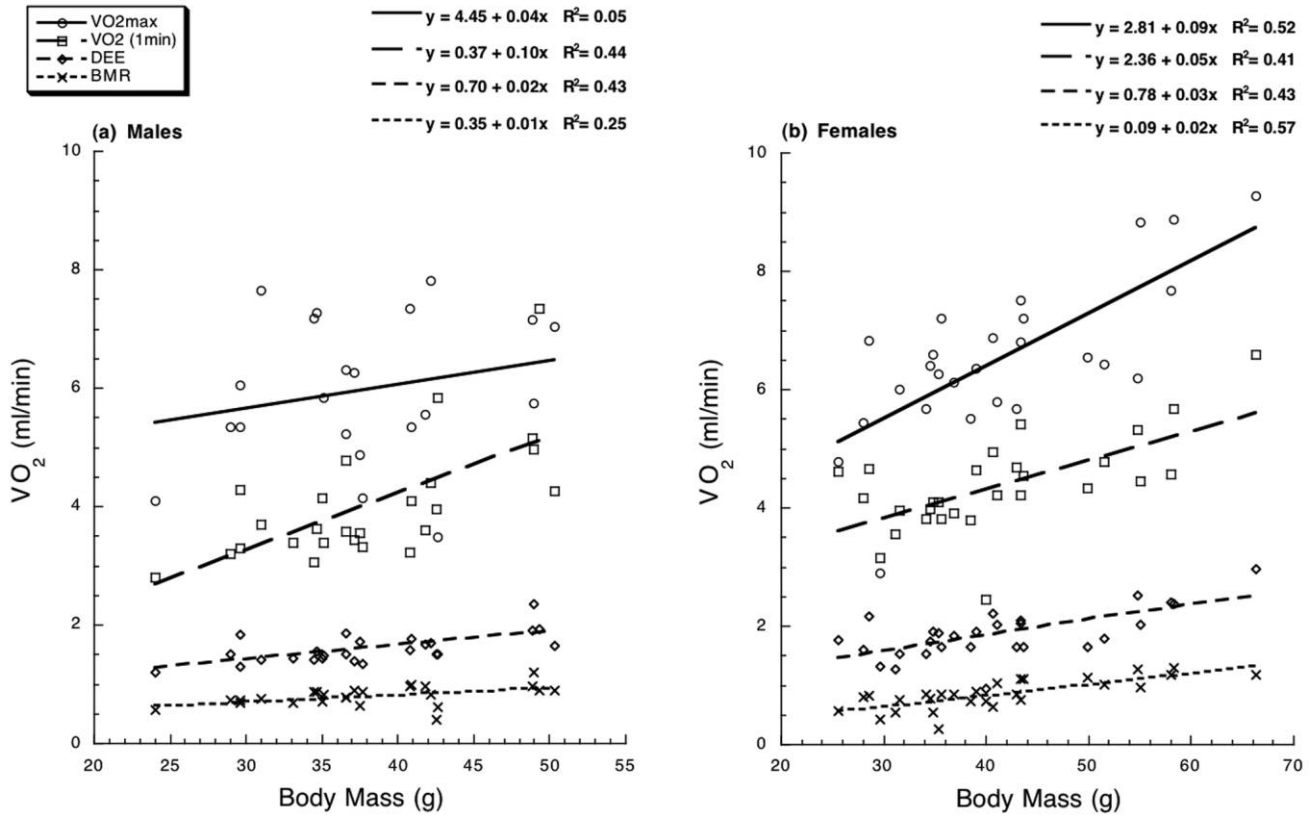

Figure 2. Graphical representation of basal metabolic rate (BMR), daily energy expenditure (DEE), $\dot{V}O_{2\,(1\,min)}$, and $\dot{V}O_{2\,(max)}$ relative to body mass in male (*a*) and female (*b*) California mice. Like other rodents that have been studied with similar methods (Chappell et al. 2004, 2007; Rezende et al. 2005, 2006, 2009; Dlugosz et al. 2009), these mice tend not to run voluntarily at power outputs that push metabolic rate near $\dot{V}O_{2\,(max)}$.

to be an important component of daily energy budgets. Voluntary activity in laboratory settings may reflect ecologically relevant daily movement patterns in the wild (Chappell et al. 2004). Intraspecific rodent studies (Chappell et al. 2004, 2007; Rezende et al. 2006) often reveal correlations among measures of voluntary activity. They also frequently show that individual variation in locomotor behavior is mechanistically related to certain suborganismal traits or to whole-animal metabolism and performance, although there is little consistency among species. Like other rodents, *P. californicus* demonstrates a number of significant correlations among voluntary-activity measurements (table A1). However, these apparently differ between the sexes.

Forced-exercise $\dot{V}O_{2\,(max)}$ is an indicator of maximum cardiopulmonary and musculoskeletal function, and as reported for other rodents (Chappell et al. 2004, 2007; Rezende et al. 2005), $\dot{V}O_{2\,(max)}$ values in California mice are well above maximum voluntary $\dot{V}O_2$ for both sexes. This indicates that routine running is not done at the highest aerobic speeds, as predicted if animals are trying to minimize the cost of transport (fig. 2; Rezende et al. 2005; Dlugosz et al. 2009). The relative mass of an organ may indicate its contribution to overall metabolic rate (Jones 1998), and in both male and female *P. californicus*, we found a number of correlations among organ masses as well as between organ masses and other traits. However, we found little consistency between males and females in correlations of activity with suborganismal traits; such inconsistency between the sexes is also typical for similar analyses in interspecific comparisons (Chappell et al. 2007).

Limitations

At first glance, it seems surprising that CORT appears to be unrelated to the majority of the metabolic and behavioral traits we measured. The lack of association may stem from our exclusive use of circulating CORT levels as the index of CORT function, which may not fully represent the physiological activity of this hormone. In general, the bioactivity of CORT is determined not only by CORT concentrations in the circulation but also by levels of corticosteroid-binding globulin (CBG) in the blood, the number and affinity of corticosteroid receptors in target tissues, and the availability of coactivator proteins within target cells. Our assay measured total CORT; thus, we could not quantify bound (attached to CBG) and unbound (free) CORT levels. Although no study has investigated whether *P. californicus* produces CBG, studies from other rodents suggest that CBG is present in this lineage (Taymans et al. 1997; Malisch et al. 2008). Future studies measuring CORT receptor density

and affinity, CBG levels, or free-plasma CORT concentrations are vital for understanding biologically meaningful individual variation in glucocorticoid levels.

Finally, although we made every effort to minimize physical and psychological stress to the mice during data collection, it is possible that voluntary exercise, $\dot{V}O_{2(max)}$, and BMR measurements may have been stressful in and of themselves and therefore may have influenced subsequent measurements. This is especially relevant because activity and metabolic data were collected in back-to-back measurements with virtually no recovery time in between. Although we believe that our results are valid, this possibility could help explain the absence of expected correlations. In addition, although combined data from previous studies (Chauke et al. 2011; Harris et al. 2011) suggest that $CORT_{AM}$ is repeatable within individuals, no data are available on repeatability of $CORT_{PM}$ within individuals. If basal $CORT_{PM}$ levels are highly variable (and therefore less repeatable) within individuals, then fewer correlations between CORT and performance would be expected (Careau and Garland 2012).

Summary and Conclusions

Hormone profiles may influence the evolution of a number of adaptations that affect locomotor activity as well as other ecologically relevant behaviors (DeVries et al. 1995; Creel et al. 1997; Miles et al. 2007; Moore and Hopkins 2009; Careau and Garland 2012). Our fundamental question involved the relationship between individual variation in circulating CORT concentrations and voluntary locomotor activity, as measured by wheel running. We expected to find substantial and statistically significant linkages between CORT levels and voluntary locomotor behaviors (e.g.. run time, number of running bouts, postural cost) and positive correlations between CORT and aerobic performance limits ($\dot{V}O_{2(max)}$ and BMR). In addition, we expected to find a fairly large number of significant relationships between CORT and lower-level traits or morphological variables (e.g.. musculoskeletal mass; Almon et al. 2008) that possibly contribute to whole-organism performance or behavior. Contrary to these predictions, we found few statistically significant correlations between CORT levels and behavioral, metabolic, or morphological variables; moreover, the few significant relationships that we found between CORT levels and performance measures were negative and differed between the sexes.

Undoubtedly, an organism's ability to adjust CORT levels is important, and both locomotion and aerobic performance are likely to be critical for survival. Our results, although unexpected, serve to demonstrate the complexity of links between hormone levels and physical activity (Guimont and Wynne-Edwards 2006; Moore and Hopkins 2009; Blumstein et al. 2010; Careau and Garland 2012).

Acknowledgments

We would like to thank Dr. Miyetani Chauke and Dr. Trynke R. de Jong for assistance with blood sampling and the UCR Biology machine shop for constructing the wheel enclosures, environmental cabinets, and treadmill. We also thank three anonymous reviewers and Dr. Theodore Garland Jr. for helpful comments on earlier drafts of this manuscript. This research was funded in part by National Institutes of Health grant R21 MH087806 to W.S.

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