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Influence of corticosterone on growth, home-cage activity, wheel running, and aerobic capacity in house mice selectively bred for high voluntary wheel-running behavior

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ABSTRACT

Glucocorticoids, a class of metabolic hormones, impact a wide range of traits (e.g., behavior, skeletal growth, muscle maintenance, glucose metabolism), and variation in concentrations of circulating glucocorticoids (such as corticosterone), at the level of natural individual variation, in relation to endocrine disorders, or from exogenous supplementation, have manifold effects. Changes in circulating corticosterone concentrations can also impact multiple aspects of locomotor behavior, including both motivation and physical ability for exercise. To examine further the role of corticosterone in locomotor behavior and associated traits, we utilized laboratory house mice from a long-term experiment that selectively breeds for high levels of voluntary exercise. As compared with four non-selected control (C) lines, mice from the four replicate High Runner (HR) lines have ~2-fold higher baseline circulating corticosterone concentrations as well as ~3-fold higher voluntary wheel running on a daily basis, higher home-cage activity when deprived of wheels, higher maximal aerobic capacity, and smaller body size; potentially, all of these differences could be modulated by circulating corticosterone. We administered 50 μg/mL corticosterone-21-hemisuccinate in the drinking water of both HR and C male mice from weaning through ~8 weeks of age. As compared with mice from C lines, HR mice had higher endogenous corticosterone levels; higher daily wheel-running distance, duration, and speed; higher maximal oxygen consumption during forced exercise (VO_2max); spent more time in the closed arms of an elevated plus maze; and had larger reproductive fat pads. For both HR and C mice, corticosterone treatment strongly suppressed endogenous circulating corticosterone levels, decreased growth rate and adult body mass, increased food and water consumption (both adjusted for body mass), increased entries into closed arms of an elevated plus maze, decreased home-cage activity (total and average intensity), decreased wheel-running distance and maximum speed, and decreased VO_2max. At the suborganismal level, corticosterone treatment decreased relative adrenal, liver, and triceps surae muscle mass, as well as tail length, but increased both subdermal and reproductive fat pad masses, as well as hematocrit. Overall, the responses of both HR and C mice to corticosterone supplementation were "negative" from a health perspective. These results have significant implications for understanding both the evolution of baseline corticosterone levels and stress-related effects on activity levels. They also suggest that patients experiencing extended periods of glucocorticoid treatment might benefit from attempts to increase their physical activity as an adjuvant.

1. Introduction

In vertebrates, variations in circulating concentrations of hormones are involved in regulation of virtually all aspects of organismal function and behavior [1–3]. The regulation and function of hormones are likely key targets of natural and sexual selection [4,5], most apparent when addressing issues of reproductive fitness [6]. However, locomotion as a function of endocrine activity has been less frequently studied, despite its importance in vertebrate survival and other aspects of Darwinian fitness [7–12].

One class of hormones with potentially far-reaching effects on locomotor physiology and behavior are the glucocorticoids. Glucocorticoids are metabolic hormones and include both cortisol and corticosterone (CORT); these steroid hormones are released by the adrenal glands and have receptors throughout the body, including the hippocampus, liver, and muscle tissues [1], all of which are involved in

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locomotor behavior. Circulating concentrations of CORT and other glucocorticoids increase in response to stress, exercise, and food intake, but are also secreted continuously on a diel rhythm by the adrenal gland [13,14]. This basal or baseline CORT impacts various aspects of physiology, including skeletal muscle function, energy balance, and glucose mobilization from the liver [15–17]. Numerous animal studies of CORT describe the hormone as a proxy measurement for the overall health of the system: circulating CORT levels described as "high" are thought to be indicative of a state of stress, famine or low health [18]. Food restriction and cold weather during sensitive periods (e.g. molting) result in raised CORT in birds [19–21]; CORT is also negatively correlated with fat stores during migration in Garden Warblers [22]. Individuals in a poor physical state are expected to exhibit lower capacity for locomotion, but some studies have found that higher baseline CORT is associated with increased physical activity in birds and mice [23–25]. In both mice and human beings, corticosterone/cortisol increases in response to high-intensity exercise [26,27], but individuals with hypercortisolism (Cushing's syndrome) suffer from fatigue and depression [28].

Hormonal manipulations, through implants or other ministrations, have provided a wealth of information about the potential effects of increased CORT on locomotor behavior or related traits in a variety of birds and rodents. In both Dark-eyed juncos and male tree sparrows, supplemental CORT is associated with decreased size/mass of flight muscles [29,30]. Corticosterone decreases territorial behavior in tree sparrows [31] and has fasting-dependent influence on activity levels: white-crowned and song sparrows increased perch-hopping activity when fasted and treated with CORT, but decreased activity when fed ad libitum during CORT treatment [19]. In free-ranging side-blotched lizards, CORT implants can reduce male home range size in some contexts, possibly because they are placed at a competitive disadvantage with other males [32,33].

Rodent studies frequently use CORT supplementation as a potential model for high stress [34–38]. Common and consistent findings include decreased body weight, increased fat deposits, and reduced cognitive function. In contrast, studies that focus on effects of CORT supplementation on locomotor behavior have had conflicting results. Injection of CORT (producing plasma levels mimicking stressor-induced levels) caused a significant increase in the locomotor activity of mice placed in a novel environment for 5 min [39]. Conversely, adult mice exposed to a 4-week treatment of 100 μg/mL CORT in drinking water showed decreased home-cage locomotion but increased body-mass gain and increased food consumption as compared to control mice [40]. Adrenalectomies significantly reduced wheel-running behavior in rats, and this behavior was subsequently restored with implantation of corticosterone-releasing pellets [41–43]. In those studies, CORT implant dosage was also positively associated with amount of wheel running, suggesting circulating CORT as a mechanism of control for locomotor behavior.

Selection experiments provide an advantageous setting to detect correlates of complex traits and their relationships [11,44,45]. Our research focuses on correlates, consequences, and mechanisms of locomotor activity in High Runner (HR) lines of mice that have experienced long-term selective breeding for high voluntary wheel-running behavior. Four replicate lines (populations) have been selectively bred for 84 generations, and voluntary wheel running has increased ~3-fold when compared with four non-selected-bred control (C) lines (Swallow et al. [46]; Garland et al. [47] and references within). In addition, the HR mice have evolved correlated differences in varied physiological and behavioral traits [11,48–51]. HR mice have baseline circulating CORT levels approximately twice those of C mice [25], with no significant difference in circadian levels or corticosteroid-binding globulin capacity [52].

Previously, we hypothesized that increased CORT levels evolved in these lines as one of several mechanisms to increase locomotor behavior, via effects on motivation and/or ability for wheel running [11,25]. If this hypothesis is correct, then manipulations to increase circulating CORT levels would be expected to increase voluntary wheel-running behavior in mice from non-selected control lines, but not necessarily cause further increases in running by High Runner mice because they have been at a selection limit of unknown causes for tens of generations [53,54]. Therefore, our study aims were twofold: 1) to raise circulating CORT in C mice, thus potentially causing an increase in wheel-running, and 2) to raise circulating CORT in HR mice, potentially surpassing the current selection limit at which evolutionary increases in wheel running have stalled.

2. Materials and methods

All experiments and methods were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

2.1. Experimental animals

Mice for this experiment were taken from an ongoing selection experiment that began in 1993. Eight breeding lines were created from an original base population of 224 outbred Harlan-Sprague-Dawley:ICR mice. Four control (C) lines are bred without regard to wheel running, while four High Runner (HR) lines are selectively bred based on total wheel revolutions during days 5 and 6 of a 6-day period of wheel access. Further details can be found in Swallow et al. [46].

We chose to use male mice, as female mice tend to have twice the levels of circulating CORT as males, and these levels may shift in response to stage of the estrus cycle [25,26]. One hundred male mice from generation 68 were randomly chosen from litters weaned at 21 days of age, weighed, toe-clipped for identification, and moved to individual cages (day 1 of the experiment). Cages contained ad libitum food (Harlan Teklad Laboratory Rodent Diet [W] 8604) and tap water, but no access to wheels. Throughout the study, mice were maintained on a 12-h light/dark cycle (0700–1900).

2.2. Corticosterone treatment

On day 5 of the experiment, drinking water treatment began for 100 mice (12–13 from each of the 8 lines; Fig. 1). Mice were randomly assigned treatment with 50 μg/mL corticosterone-21-hemisuccinate (Steraloids, Inc., Newport RI) solution or tap water. 50 μg/mL is the concentration of corticosterone-21-hemisuccinate in drinking water needed to increase plasma CORT to 12.5 μg/100 mL in adrenalectomized rats [55]. As the mean difference in plasma CORT between male HR and C mice is 5.4 μg/100 mL [25], mice were given 50 μg/mL in order to raise plasma CORT beyond the dampening response predicted from negative feedback within the hypothalamic-pituitary-adrenal axis (HPA; [56]). Corticosterone hemisuccinate was dissolved in tap water by increasing the pH of solution to ~10 and stirring for 12 h; pH was returned to 7.0–7.4 before pouring into drinking bottles.

2.3. Experimental procedures and measurements

Mouse mass, food mass, and water/solution mass were measured every 4 days, and water/solution was replaced every 8 days. Each cage was equipped with a passive infrared sensor to monitor home-cage activity (Warthog Systems Sensor Activity Monitor program, Mark A. Chappell, University of California, Riverside). Mouse activity was measured by the sensors 3×/s, with activity averaged into a normalized activity unit (NAU) that ranged from zero to one for each second, then summed for each minute [57,58]. Every cage was monitored overnight from 1700 to 1200 the next day (i.e., 19 h), each day of the experiment. Total home-cage activity was taken as the sum of all activity over the daily monitoring period. As previously, we also tallied the number of 1-min intervals during which any activity was registered.

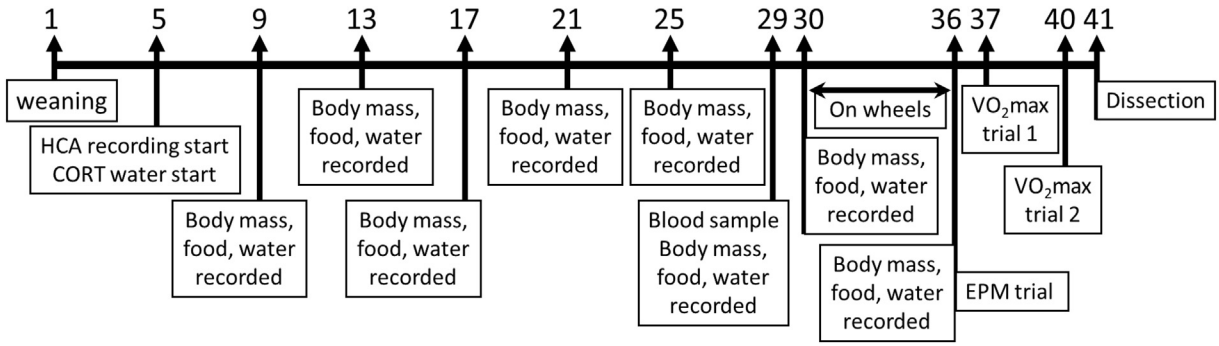

Fig. 1. Experimental timeline. Weaning occurred at 21 days of age and is designated as experimental day 1.

Finally, we divided total SPA by minutes of activity to estimate mean intensity of home-cage activity, i.e., the average amount of activity per minute when any home-cage activity was occurring. This gives a measure that is analogous to mean wheel speed (see below).

The day before mice were given wheel access (experimental day 29, time range = 1400–1530), 100 μL of blood was taken via retro-orbital puncture under isoflurane anesthesia using two heparinized micro-hematocrit tubes. All blood sampling occurred within 3 min from disturbance of the mouse [59]. Microhematocrit tube contents were collected in microcentrifuge tubes on ice until centrifuge (~20 min maximum). Blood samples were spun at 11,500 rpm for 5 min at 18 °C degrees. Plasma was stored at −80 °C until assayed.

Blood samples were assayed with a cat. no. K014 DetectX® Corticosterone Enzyme Immunoassay kit from Arbor Assays, Inc. (Ann Arbor MI). Samples were diluted 1:100 and measured in duplicate in 96-well plates. Absorbances were read at 450 nm using a Spectramax Plus 384 microplate reader with Softmax® Pro 4.3 reading software (Molecular Devices Corporation, Sunnyvale CA, USA) and compared with a standard curve generated individually for each plate. Upon request, independent testing by Arbor Assays confirmed the inability of this kit to detect corticosterone-21-hemisuccinate. Therefore, we presume the assay was detecting endogenous CORT concentrations in plasma. However, we do not have direct evidence that the corticosterone-21-hemisuccinate molecule stays intact in vivo, i.e., that it is not metabolized to CORT prior to being eliminated, so this presumption should be viewed with caution.

On day 30 after weaning (age 7 weeks), all mice were given free access to exercise wheels of the same type as used for selective breeding [46]. Wheels were attached to revolution counters that recorded total revolutions in 1-min bins for 23 h, beginning at 1300. We analyzed daily mean values for total revolutions run on days 5 and 6 of a 6-day trial, the number of minutes with at least one revolution (intervals), the average speed (total revolutions/intervals), and the single fastest interval. Mouse mass, food mass, and water/solution mass were recorded prior to wheel access and again at the end of the 6-day period.

On day 36 of the experiment (conclusion of wheel testing), mice were tested on an elevated plus maze (EPM) to examine potential changes in anxiety-like behavior [60,61]. The maze was constructed of sheets of black trovicel, cut to size, and placed on adjustable aluminum legs for a maze height of 90 cm. The black material allows video tracking software to detect white mice more easily. The maze itself consists of four arms (45 cm length × 3.5 cm width), two with 20 cm high walls ("closed arms") and two with no walls ("open arms"), and a center area (3.5 × 3.5 cm) connecting the arms. Each mouse was restricted from wheel access for 2–4 h prior to the test to allow for quick capture. It was then placed in the center of the EPM facing an open arm, and video-recorded for 5 min. Video was recorded onto a laptop computer via a Microsoft Lifecam Studio webcam, model #1425. Videos were converted to audio-video interleaved format (.avi) with Arcsoft Media Converter 8, then processed for data capture using Ethovision XT7 video tracking software (Noldus, Inc.). The program tracked time spent in open arms, closed arms, and center, as well as latency to enter open and closed arms, average velocity, and total distance moved.

The day following EPM testing (day 37), each mouse was tested for maximal oxygen consumption (VO2max; [62,63]), which is an important determinant of the capacity to sustain endurance-type activity [64–66] between 0800 and 1200. Mice were run inside a manually-controlled wheel metabolic chamber at increasing speeds until they were unable to run faster and appeared exhausted, as part of an open circuit respirometer [67]. Each mouse ran for 3.5–6 min, and the highest 60-s interval of oxygen consumption was calculated using Warthog Software (http://warthog.ucr.edu/). Testing was repeated 4 days later (day 41). Both measurements for VO_2max per mouse were analyzed, and testing was repeated a third time if the difference between the first two readings was greater than twice the mean difference between readings for all mice tested. The higher of two values was taken as the VO_2max.

After the second VO_2max test, mice were placed in regular cages with food and tap water access. Mice did not receive CORT solution during this time. On day 41, mice were sacrificed via decapitation, trunk blood was collected, and tail length, heart ventricle mass, adrenal gland masses, triceps surae mass, and testis masses were measured. Adrenal glands were frozen at −80 °C and re-weighed at a later date for higher precision. Blood was collected in heparinized micro-hematocrit tubes and centrifuged for 5 min in a micro-hematocrit centrifuge, hematocrit was recorded in duplicate, and plasma was stored at −80 °C until analysis. Carcasses were frozen at −20 °C and at a later date thawed, and liver, kidneys, reproductive fat pads (fat pads surrounding the testes), and subdermal (inguinal, gluteal, and lumbdoidal) fat pads [68] were dissected and weighed. Fat pads were not recoverable from all carcasses due to skinning methods from the first dissection.

2.4. Statistical analyses

Results were analyzed using mixed-model ANCOVAs in SAS Procedure Mixed, as in many previous studies of these lines (e.g., see [47]). Dependent variables were transformed as needed to improve normality of residuals. Effects of linetype (HR vs. C), CORT treatment, and their interaction were tested relative to the variation among lines (or the treatment * line(linetype) interaction), with 1 and 6 degrees of freedom.

Effects of the mini-muscle phenotype [69,70] were also tested relative to the residual d.f. The mini-muscle phenotype is primarily described as a ~50% reduction in the hindlimb muscle mass in two of the four HR lines; this phenotype also includes enlarged hearts, livers, kidneys, and fat pads [70–73]. In the present sample, 13 of 88 individuals expressed the mini-muscle phenotype, all of them within HR lines #3 (which has been fixed for the underlying allele for many generations) and #6 (lab designations), as expected from previous studies.

Body mass was included as a covariate when appropriate (Table 1). In separate analyses, we also tested for an interaction between mini-

Table 1
Results of analysis of covariance for traits measured in male mice from replicate High Runner and control (C) lines. For main effects (corticosterone treatment, linetype, mini-muscle status) and for body mass as a covariate, *P* values < 0.05 (or < 0.1 for interaction effects) are in bold and preceded by an asterisk (values also significant after correction for multiple comparisons by the pFDR Q-value procedure are also underlined, except for body mass as a covariate, which was not included in this correction [see Methods]). For some traits, a significant (*P* < 0.1) interaction between CORT treatment and mini-muscle status was observed (see Results and Online Supplemental Table 2): all of the results show here are for models that did not include this interaction. Categories of "1" and "0" indicate the presence or absence of a condition (CORT, HR, Mini-muscle). Where indicated, dependent variables were transformed to improve normality of residuals from the statistical model; in those cases, values for least squares means are for the transformed data.

Trait	Trans-form	N	P CORT	P linetype	P inter-action	P mini-muscle	P body mass	CORT	HR vs. C	Mini	Least squares mean	SE of LSM
Growth rate		91	***0.0006**	0.7055	0.8179	0.1173	–	0	0		0.489	0.0507
CORT*mini inter.								0	1		0.460	0.0466
								1	0		0.363	0.0508
								1	1		0.342	0.0466
										0	0.448	0.0317
										1	0.379	0.0485
Body mass at wheel start (g)		91	***0.0031**	0.4607	0.9603	0.1386	–	0	0		27.18	1.656
CORT*mini inter.								0	1		25.57	1.486
								1	0		23.98	1.660
								1	1		22.44	1.485
										0	25.98	0.998
										1	23.60	1.672
% Mass change during 6 days Wheel access		89	***0.0290**	0.7174	0.4172	0.3622	–	0	0		−1.59	0.964
								0	1		−0.61	0.783
								1	0		−3.10	0.994
								1	1		−3.45	0.789
										0	−1.65	0.415
										1	−2.73	1.080
Body mass at dissection (g)		90	***0.0011**	0.3918	0.7092	0.1101		0	0		27.50	1.632
CORT*mini inter.								0	1		25.96	1.485
								1	0		23.63	1.636
								1	1		21.53	1.481
										0	25.86	0.988
										1	23.45	1.596
Food consumption days 21–25 (g/day)		90	***0.0080**	0.3725	***0.0784**	***0.0031**	< 0.0001	0	0		4.79	0.145
								0	1		5.11	0.122
								1	0		5.33	0.142
								1	1		5.31	0.127
										0	4.89	0.076
										1	5.38	0.153
Food consumption days 25–29 (g/day)		91	***0.0089**	0.3994	0.1762	0.0715	< 0.0001	0	0		4.40	0.208
								0	1		4.78	0.181
								1	0		5.05	0.208
								1	1		5.09	0.188
										0	4.63	0.115
										1	5.02	0.214
Food consumption during wheel access (g)		90	0.6856	0.1287	***0.0441**	0.8071	< 0.0001	0	0		4.73	0.249
								0	1		5.34	0.206
								1	0		5.12	0.251
								1	1		4.86	0.206
										0	4.93	0.111
										1	5.09	0.246
Water consumption days 21–25 (g/day)	^0.5	89	***0.0041**	0.9878	0.8188	***0.0178**	< 0.0001	0	0		2.756	0.2031
								0	1		2.731	0.1894
								1	0		3.307	0.2043
								1	1		3.340	0.1932
										0	2.843	0.1195
										1	3.224	0.1785
Water consumption days 25–29 (g/day)	^0.5	90	***0.0078**	0.8233	0.7372	***0.0304**	< 0.0001	0	0		2.676	0.2139
								0	1		2.682	0.2015
								1	0		3.219	0.2148
								1	1		3.328	0.2046
										0	2.804	0.1223
										1	3.149	0.1794
Water consumption during wheel access (g/day)	^0.5	86	***0.0086**	0.9090	0.2837	0.0563	0.0030	0	0		2.762	0.1583
CORT*mini inter.								0	1		2.886	0.1367
								1	0		3.245	0.1578
								1	1		3.162	0.1414
										0	2.853	0.0859
										1	3.175	0.1640

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Table 1 (*continued*)

Trait	Trans-form	N	P CORT	P linetype	P inter-action	P mini-muscle	P body mass	CORT	HR vs. C	Mini	Least squares mean	SE of LSM
Home-cage activity (arbitrary units) (d 21–25)	^0.3	91	***0.0339**	0.0808	0.7233	0.5234	–	0	0		3.930	0.2150
								0	1		4.438	0.1763
								1	0		3.665	0.2158
								1	1		4.091	0.1755
										0	4.118	0.1114
										1	3.944	0.2533
Home-cage intensity (activity/min) (d 21–25)	^0.5	91	***0.0206**	0.1256	0.7124	0.4277	–	0	0		0.2412	0.02380
								0	1		0.2879	0.01940
								1	0		0.2007	0.02395
								1	1		0.2360	0.01930
										0	0.2536	0.01152
										1	0.2293	0.02810
Home-cage activity (arbitrary units) (d 25–29)	^0.5	91	0.0628	0.1222	0.4280	0.2694	–	0	0		9.70	1.000
								0	1		12.04	0.833
								1	0		9.05	1.003
								1	1		10.62	0.830
										0	11.03	0.544
										1	9.68	1.158
Home-cage intensity (activity/min) (d 25–29)	^0.5	91	***0.0481**	0.1879	0.6185	0.1760	–	0	0		0.4775	0.03003
CORT*mini inter.								0	1		0.5324	0.02476
								1	0		0.4488	0.03013
								1	1		0.4883	0.02467
										0	0.5122	0.01590
										1	0.4613	0.03516
CORT (pg/mL)	$\log_{10}$	75	* < **0.0001**	***0.0083**	0.9273	0.5501		0	0		4.896	0.1214
								0	1		5.249	0.0878
								1	0		3.905	0.1127
								1	1		4.274	0.1042
										0	4.619	0.0490
										1	4.544	0.1165
Wheel revolutions days 5–6 (per day)		87	0.1096	* < **0.0001**	0.1036	***0.0018**	–	0	0		1245	725.5
CORT*mini inter.								0	1		8747	595.1
								1	0		1263	741.5
								1	1		7058	600.3
										0	6064	361.8
										1	3092	851.3
Intervals (min/day)		87	0.3116	***0.0068**	0.4624	0.0839	–	0	0		278.5	45.947
CORT*mini inter.								0	1		495.59	38.1719
								1	0		271.34	46.6316
								1	1		453.31	38.3495
										0	424.18	24.7256
										1	325.19	53.5285
Average speed (revs/min)		87	0.1287	***0.0005**	0.2890	0.0582	–	0	0		6.02	1.452
								0	1		17.31	1.203
								1	0		5.59	1.470
								1	1		15.23	1.208
										0	12.74	0.778
										1	9.21	1.689
Max speed (revs/min)		87	0.0604	***0.0003**	0.7018	0.0751	–	0	0		16.00	1.817
								0	1		30.03	1.513
								1	0		13.62	1.844
								1	1		26.64	1.523
										0	23.55	0.879
										1	19.59	2.043
VO_2max (mL O_2/min)		87	***0.0097**	***0.0393**	0.1683	0.3727	< 0.0001	0	0		4.72	0.143
								0	1		5.22	0.116
								1	0		4.49	0.141
								1	1		4.78	0.125
										0	4.72	0.073
										1	4.88	0.163
EPM latency to Enter open arm	$\log_{10}$(value +1)	78	0.4036	0.2615	***0.0267**	0.4541	–	0	0		0.43	0.217
								0	1		1.18	0.187
								1	0		1.10	0.239
								1	1		0.82	0.187
										0	0.99	0.095
										1	0.78	0.262

(*continued on next page*)

Table 1 (*continued*)

Trait	Trans-form	N	P CORT	P linetype	P inter-action	P mini-muscle	P body mass	CORT	HR vs. C	Mini	Least squares mean	SE of LSM
EPM % time in closed arms	^0.3	80	0.8050	***0.0494**	0.2316	0.3982	–	0	0		3.08	0.119
								0	1		3.48	0.102
								1	0		3.24	0.122
								1	1		3.38	0.104
										0	3.24	0.055
										1	3.35	0.126
EPM entries into closed arms		80	***0.0285**	0.5488	0.5769	***0.0067**	–	0	0		19.3	1.94
								0	1		21.3	1.63
								1	0		24.7	2.10
								1	1		24.9	1.70
										0	19.0	0.86
										1	26.0	2.30
Tail length (mm)		91	***0.0020**	0.2099	0.9744	0.1440	0.0094	0	0		77.4	2.18
								0	1		80.7	1.84
								1	0		69.9	2.16
								1	1		73.2	1.97
										0	77.1	1.15
										1	73.6	2.31
Left adrenal (g)		81	***0.0309**	0.4894	0.6083	0.8456	0.1173	0	0		0.00452	0.000464
								0	1		0.00499	0.000349
								1	0		0.00357	0.000432
								1	1		0.00369	0.000397
										0	0.00414	0.000190
										1	0.00424	0.000474
Mean kidney (g)		86	0.5904	0.4235	***0.0897**	0.9054	< 0.0001	0	0		0.2016	0.00862
								0	1		0.2191	0.00702
								1	0		0.2148	0.00853
								1	1		0.2123	0.00766
										0	0.2113	0.00430
										1	0.2125	0.00981
Ventricle (g)		89	0.3413	0.0847	0.1014	***0.0319**	< 0.0001	0	0		0.1117	0.00333
								0	1		0.1220	0.00265
								1	0		0.1127	0.00323
								1	1		0.1165	0.00289
										0	0.1114	0.00166
										1	0.1200	0.00370
Liver (g)	$\log_{10}$	87	***0.0300**	0.7939	0.2017	* < **0.0001**	< 0.0001	0	0		0.1660	0.01843
								0	1		0.1589	0.01463
								1	0		0.1211	0.01793
								1	1		0.1387	0.01583
										0	0.0969	0.00942
										1	0.1955	0.02051
Mean testis (g)		87	0.1829	0.1168	0.9149	0.5996	0.2787	0	0		0.1110	0.00744
								0	1		0.0942	0.00687
								1	0		0.1064	0.00744
								1	1		0.0890	0.00707
										0	0.0985	0.00466
										1	0.1017	0.00690
Subdermal fat pad (g)		84	***0.0072**	0.4539	0.9359	***0.0076**	< 0.0001	0	0		0.2586	0.04661
								0	1		0.2181	0.03856
								1	0		0.3931	0.04553
								1	1		0.3574	0.04135
										0	0.2391	0.02346
										1	0.3745	0.04746
Reproductive fat pad (g)	$\log_{10}$	72	***0.0045**	***0.0401**	***0.0959**	***0.0049**	< 0.0001	0	0		−0.7578	0.06687
								0	1		−0.8123	0.05056
								1	0		−0.3963	0.06470
								1	1		−0.6454	0.05584
										0	−0.7584	0.02693
										1	−0.5475	0.06583
Subdermal/reproductive fat pad	$\log_{10}$	69	0.1410	0.2060	***0.0368**	0.8214	< 0.0001	0	0		0.1732	0.06136
								0	1		0.163	0.04653
								1	0		0.00108	0.06099
								1	1		0.1796	0.05096
										0	0.1209	0.02829
										1	0.1376	0.06774
Mean tricep surae muscle (g)		88	***0.0052**	0.5754	0.3872	* < **0.0001**	< 0.0001	0	0		0.1109	0.00464
CORT*mini inter.								0	1		0.1051	0.00379
								1	0		0.8981	0.00445
								1	1		0.0908	0.00412
										0	0.1223	0.00197
										1	0.0760	0.00462

(*continued on next page*)

Table 1 (*continued*)

Trait	Trans-form	N	P CORT	P linetype	P inter-action	P mini-muscle	P body mass	CORT	HR vs. C	Mini	Least squares mean	SE of LSM
Hematocrit at dissection (g)		84	***0.0116**	0.1033	0.3997	0.5438	–	0	0		47.28	1.158
								0	1		50.15	0.946
								1	0		50.46	1.196
								1	1		52.05	0.952
										0	50.43	0.582
										1	49.54	1.347

* indicates P < 0.05. * plus underlining indicates still significant after correction for multiple comparisons.

muscle status and CORT treatment (Online Supplemental Table 2): this interaction effect was statistically significant ($P < 0.1$) in 12 of 59 models analyzed. When significant, we discuss results from these full models; otherwise, we discuss results of models that did not include the CORT*mini interaction.

For main effects, $P < 0.05$ was considered statistically significant, whereas interactions were considered significant when $P < 0.1$ because ANOVAs generally have relatively lower power to detect interactions (see [74,75] and following commentaries). To control the number of false positives (i.e., rejecting true null hypotheses), we used the Positive False Discovery Rate Procedure as implemented in SAS Procedure MULTTEST. We did this using the P values presented in Table 1. We excluded P values for body mass when used as a covariate because this was not of primary interest in the present study and instead is essentially a nuisance variable when examining effects of the main experimental factors (CORT treatment and linetype) on such traits as organ sizes. Analysis of Q values for the remaining 128 P values in Table 1 indicated that a P value of ~0.0237 could be considered significant for main effects (rather than the nominal 0.05) and that a P value of ~0.0768 (rather than the nominal 0.1) could be considered significant for the CORT*linetype interaction term. For simplicity, all P values referred to in the text are the nominal values, uncorrected for multiple comparisons, unless otherwise stated.

3. Results

Table 1 presents results from the ANCOVAs for key traits, including least squares means and associated standard errors by groups. Results for all traits can be found in Online Supplemental Table 1 and Online Supplemental Table 2 (models with CORT*mini interactions).

3.1. Body mass and growth

Groups did not differ in body mass at weaning, i.e., before treatment began (Online Supplemental Table 1). Administration of CORT in drinking water significantly decreased growth rate (change in body mass from 3 to 7 weeks of age) for both HR and C mice (Fig. 2A: $P = 0.0051$), in a model that included a significant CORT*mini-muscle interaction ($P = 0.0129$), indicating that CORT did not significantly reduce growth rate for mini-muscle individuals. Adult body mass at the start of the 6-day wheel-running trial tended to be reduced by CORT ($P = 0.0793$), with a significant CORT*mini interaction ($P = 0.0033$), indicating that CORT had less of a negative effect on mini-muscle individuals. Results were similar for body mass taken the day before dissections (Fig. 2B: CORT $P = 0.0125$; CORT*mini $P = 0.0023$). In addition, CORT treatment resulted in a higher percent body mass loss during the first 6 days of wheel access (Fig. 2C: $P = 0.0290$).

3.2. Food and water consumption

We analyzed food and water consumption during the 8 days immediately prior to adult wheel running, as this is the period when mice received the longest treatment without consumption measures potentially confounded by high levels of physical activity on wheels (e.g., see [57]). Administration of CORT significantly increased mass-independent food consumption on days 21–25 and 25–29 ($P = 0.0080$ and $P = 0.0089$, respectively), and the effect on C mice was greater than for HR mice during the first time period (interaction $P = 0.0784$) (Table 1). In addition, mini-muscle individuals consumed more food ($P = 0.0031$ and $P = 0.0715$, respectively). Adding the amount of home-cage activity, its duration, and/or its intensity to the statistical models indicated that these activity measures were positively but not significantly predictive of food consumption, with little effect of the significance levels of the main effects and interaction (results not shown).

During wheel access CORT treatment, which was continuing, increased food consumption in C mice while decreasing it in HR mice (interaction $P = 0.0441$).

Corticosterone treatment significantly increased mass-independent water consumption of both C and HR mice in the 8 days prior to wheel access ($P = 0.0041$ and $P = 0.0078$, respectively), and mini-muscle mice also consumed more water than normal-muscled individuals ($P = 0.0178$ and $P = 0.0304$, respectively). The effects of CORT remained statistically significant ($P = 0.0095$ and $P = 0.0180$, respectively) after including food consumption as an additional covariate (effect of food consumption: $P = 0.0018$ and $P < 0.0001$, respectively).

During the 6-day period of wheel access, CORT treatment increased water consumption ($P = 0.0086$). When food consumption was included as a covariate (food $P < 0.0001$), the overall effect of CORT treatment remained highly significant ($P = 0.0003$) and an interactive effect of CORT and mini-muscle status was revealed ($P = 0.0015$; Online Supplemental Table 2): mini-muscle mice receiving CORT increased their drinking more than other mice. Incorporating the amount of running on days 5 and 6 as an additional covariate indicated a positive effect on water consumption (running $P = 0.0135$), with the effects of food consumption ($P < 0.0001$), CORT ($P = 0.0007$), and the CORT*mini interaction ($P = 0.0047$) remaining significant (Online Supplemental Table 2).

3.3. Home-cage activity

Home-cage activity in the 8 days prior to wheel access (experimental days 21–25 and 25–29) was lower in groups receiving CORT ($P = 0.0339$ and $P = 0.0628$, respectively), but did not differ significantly between HR and C mice. The CORT-induced reductions in activity were caused by reductions in the average intensity of activity per minute ($P = 0.0206$, and $P = 0.0481$, respectively), not the amount of time spent in cage activity ($P = 0.3468$, and $P = 0.4217$, respectively). During the period of wheel access, home-cage activity was reduced for all groups by nearly 50% and did not differ between linetypes ($P = 0.7016$) or CORT treatment groups ($P = 0.6765$).

3.4. Plasma corticosterone

As mentioned above, the assay used did not detect corticosterone-21-hemisuccinate, so results reflect endogenous CORT. Mice receiving CORT had much lower baseline plasma CORT concentrations than those

receiving tap water (Table 1, Fig. 3: $P < 0.0001$), and HR mice had higher CORT than those from C lines ($P = 0.0083$).

3.5. Voluntary wheel-running behavior

As expected, the effect of linetype was highly statistically significant for average distance run on days 5 and 6 of the 6-day period of wheel access, as well as time spent running (1-min intervals with any revolutions), mean speed (distance/intervals), and maximum speed (Table 1; Fig. 4); HR mice ran more, longer, and faster. The mini-muscle phenotype was negatively related to all four metrics of wheel running (Table 1), with the effect reaching statistical significance for distance run ($P = 0.0018$). CORT treatment generally decreased all measures of wheel running, although with some interactive effects (Table 1).

The CORT*mini interaction for revolutions/day ($P = 0.0852$) and for intervals/day ($P = 0.0921$) indicated that mini-muscle individuals experienced a substantially greater decrease than other mice (Online Supplemental Table 2). In these full models, for revolutions/day and for maximum speed, the main effect of CORT treatment was also significant ($P = 0.0395$ and $P = 0.0438$, respectively), indicating that CORT decreased daily running distance and maximum speed for all groups of mice. Inspection of the least squares means from the full models indicated that the decrease in average daily wheel-running was caused by reductions in both the amount of time spent running and in average running speeds (Online Supplemental Table 2).

3.6. Maximal oxygen consumption

VO_2max was higher in HR than C mice ($P = 0.0393$) (Fig. 5). Corticosterone treatment significantly reduced VO_2max in both C and HR mice ($P = 0.0097$), with no significant interaction ($P = 0.1683$). Both HR and C mice receiving CORT had higher hematocrit at the time of dissection ($P = 0.0116$). When hematocrit was added to the statistical model for VO_2max it was not a significant predictor ($P = 0.2991$), and the P values for CORT treatment and linetype changed very little (Online Supplemental Table 1). We also tested for interactions between hematocrit and CORT administration, linetype, and both of these factors (i.e., both 2-way interactions and 3-way interaction; [66]), and none of these were statistically significant (results not shown).

When heart ventricle mass was added to the statistical model for VO_2max it was not a significant predictor ($P = 0.6427$), and the P values for CORT treatment and linetype changed little (Online Supplemental Table 1). However, in a model that included interactions between ventricle mass and the main effects, the 3-way interaction of ventricle mass, linetype and CORT treatment was statistically significant ($P = 0.0482$). In this model the main effects of CORT ($P = 0.3600$) and linetype ($P = 0.5979$) were non-significant, with their interaction having a P value of 0.0914. Based on a ln maximum likelihood ratio test, the more complicated model fit the data significantly better than the reduced model (χ^2 with 4 d.f. = 35.7, $P < 0.000001$). These results suggest that the effect of heart size on maximal aerobic capacity varies between HR and C mice and/or in relation to CORT supplementation.

3.7. Elevated plus maze

Trial duration varied somewhat (due to mouse jumping or human error; range = 260–300 s) so we either examined percentages or used total time as a covariate in analyses. Mice spent ~half the time in the maze moving (142 ± 28.4 s) and traveled on average 10.3 ± 0.31 m (range: 4.0–29.4 m). As expected, mice spent more time in the closed arms (159 ± 51.0 s) than the open arms (54 ± 45.1 s) or center area (85 ± 33.7 s). Two mice did not enter the open arms at any point.

A total of 13 aspects of behavior in the EPM were analyzed (Online Supplemental Table 1); of these, three showed evidence of main effects or interactions. Corticosterone treatment increased the number of entries into closed arms of the elevated plus maze (Fig. 6A: $P = 0.0285$), and mini-muscle individuals had more entries into closed arms ($P = 0.0067$) when compared to other mice. Mice from HR lines spent more time in closed arms (~62%) as compared with C mice (~49%) (Fig. 6B: $P = 0.0494$). The interaction between CORT and linetype was significant for the latency to enter an open arm (Table 1: $P = 0.0267$), with the primary effect being that CORT increased the latency for C mice. Post-hoc comparison of the Least Squares Means indicated that, for mice without CORT, HR lines took longer to enter an open arm ($P = 0.0237$), but for mice with CORT this difference was not significant ($P = 0.3392$).

3.8. Organ masses

With body mass as a covariate, CORT treatment affected the size of several organs (Fig. 7; Table 1). Both subdermal and reproductive fat pad masses were significantly greater in CORT treatment groups ($P = 0.0072$ and $P = 0.0045$, respectively). The reproductive fat pad mass of HR and C mice responded somewhat differently to CORT treatment (interaction $P = 0.0959$; Table 1), with a greater increase in fat pad size in C mice (Fig. 7D). (As shown in Table 1, sample size was smaller for measurements of fat pads, as these measurements were part of a second dissection and some fat pads were lost during initial skinning.)

To explore possible redistribution of body fat, we also analyzed the ratio of SDFP/RFP, log-transformed to reduce positive skewness of residuals and with body mass as a covariate. For this ratio, we find a highly significant negative effect of body mass ($P < 0.0001$) and a significant interaction between CORT treatment and linetype ($P = 0.0368$). (Results for the interaction were similar if body mass was not included as a covariate.) Examination of the LS Means showed that mice from Control lines that were administered CORT had lower values than the other three groups (Table 1).

Liver and muscle masses were lower in mice receiving CORT treatment ($P = 0.0300$ and $P = 0.0052$, respectively), but ventricle and testis mass were unaffected. The interaction effect was nominally significant for kidney mass: C mice with tap water had smaller kidneys than all other groups (interaction $P = 0.0897$; Table 1). Mice receiving supplemental CORT also had shorter tails as adults ($P = 0.0020$; Table 1) and smaller adrenal glands ($P = 0.0309$; Table 1).

HR mice had smaller reproductive fat pads at dissection when compared to C mice ($P = 0.0401$; with body mass as a covariate). Mini-muscle mice had significantly larger reproductive and subdermal fat pads, livers, and heart ventricles (Table 1). As expected, mini-muscle individuals had smaller triceps surae muscles, with a significant interactive effect of CORT treatment, such that these individuals were less negatively affected by CORT treatment (Online Supplemental Table 2).

4. Discussion

Because the assay that we used could not detect corticosterone hemisuccinate, we do not know the total circulating CORT concentration (endogenous + exogenous) in our experimental animals. However, as shown in Fig. 3, plasma CORT concentrations were strongly suppressed in animal receiving exogenous corticosterone hemisuccinate in drinking water. Moreover, several phenotypes that can be viewed as diagnostic of high circulating CORT levels, e.g., reduced growth rates, muscle mass, and adrenal mass, were affected as would be expected based on the literature. Beyond this, we did not observe increased mortality in the CORT-treated groups, nor did we observe aberrant behaviors or obvious signs of lethargy or sickness. Therefore, we believe that circulating CORT concentrations likely were within physiological ranges.

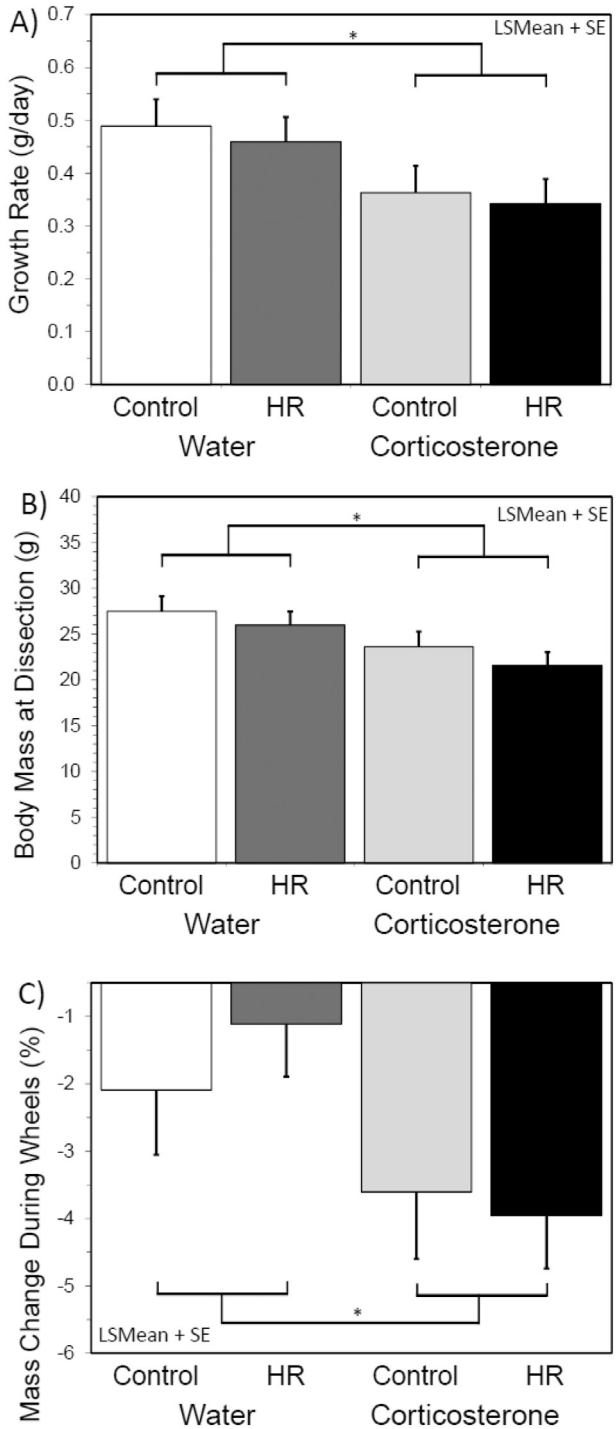

Fig. 2. Least squares means and standard errors for HR and C lines, with CORT solution or tap water treatment, for (A) growth rate measured as change in body mass from weaning until beginning of wheel testing at ~7 weeks of age, (B) body mass the day before dissection (~9 weeks of age), and (C) percent body mass lost during wheel testing over 6 days. * indicates $P < 0.05$. Corticosterone in drinking water significantly suppressed growth rate (A: $P = 0.0006$, Table 1) and hence adult body mass at sacrifice (C: $P = 0.0011$, Table 1). In addition, when adult mice were given wheel access for six days, those with CORT in the drinking water lost significantly more body mass on an absolute basis ($P = 0.0480$) and as a percent of starting body mass (C: $P = 0.0290$, Table 1). [Note that for growth rate and body mass the CORT*-mini-muscle interaction was significant ($P = 0.0129$ and $P = 0.0023$, respectively), but values shown here are from simplified models that did not include this term.]

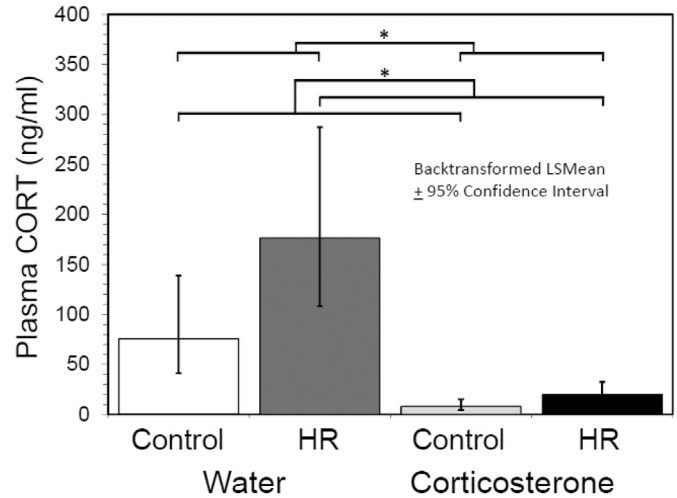

Fig. 3. Least squares means and 95% confidence intervals for HR and C lines, with CORT solution or tap water treatment, for plasma CORT concentrations from blood samples taken immediately prior to adult wheel testing (note that the assay did not detect supplemental corticosterone hemisuccinate received from drinking water). Statistical analyses were performed on log-transformed values (Table 1). On the backtransformed scale, HR mice have > 2× circulating CORT as compared with C mice. In addition, corticosterone treatment greatly reduced the amount of detectable corticosterone in plasma. * indicates $P < 0.05$.

4.1. Voluntary wheel-running behavior

As expected from numerous previous studies, mice from the selectively bred High Runner lines ran much more than those from the non-selected C lines (Fig. 4). Contrary to our hypothesis, however, corticosterone supplementation did not increase daily wheel-running distance in either HR or C mice. Instead, daily running distance tended to be decreased in both groups (Fig. 4A), which was related to smaller decreases in both the duration and the average and maximum speed of running (Fig. 4A–D). Although statistical analyses were not performed and samples sizes were small, a 1954 paper concluded that "cortisone and hydrocortisone increases voluntary [wheel running] activity when given subcutaneously and orally in normal, castrated, as well as in adrenalectomized male and female rats" [76]. Adrenalectomies in male rats decreases wheel-running behavior [77], whereas CORT administered to adrenalectomized males restores wheel-running (via implant: [41],via injection:[42]). Further investigation with varying doses of CORT showed a positive dose-dependent relationship between CORT and wheel-running behavior [43]. Mice in the current study were not adrenalectomized, rats from the above-mentioned studies were adults, and, of course, mice are not rats, so results are not directly comparable. In any case, further studies of the effects of exogenous glucocorticoids on voluntary exercise are clearly warranted.

4.2. Maximal oxygen consumption and lower-level correlates

Previous studies have shown that mice from the High Runner lines have higher VO_2max than those from control lines [53,67,70]. In addition, HR mice – but not C mice – sometimes run voluntarily on wheels at or near their maximal aerobic speed (speed at which VO2max is obtained; [78,65]). Thus, the reduction in VO_2max caused by CORT administration would be expected to cause at least some reduction in wheel running by HR mice, but not necessarily by mice from C lines. That expectation is consistent with our results, as the decrease in daily running distance caused by exogenous CORT tended to be greater for HR mice (1687 revs/day or 19%) than C mice (114 revs/day or 9%) (interaction $P = 0.1154$).

Some of the lower-level traits we measured are known to affect

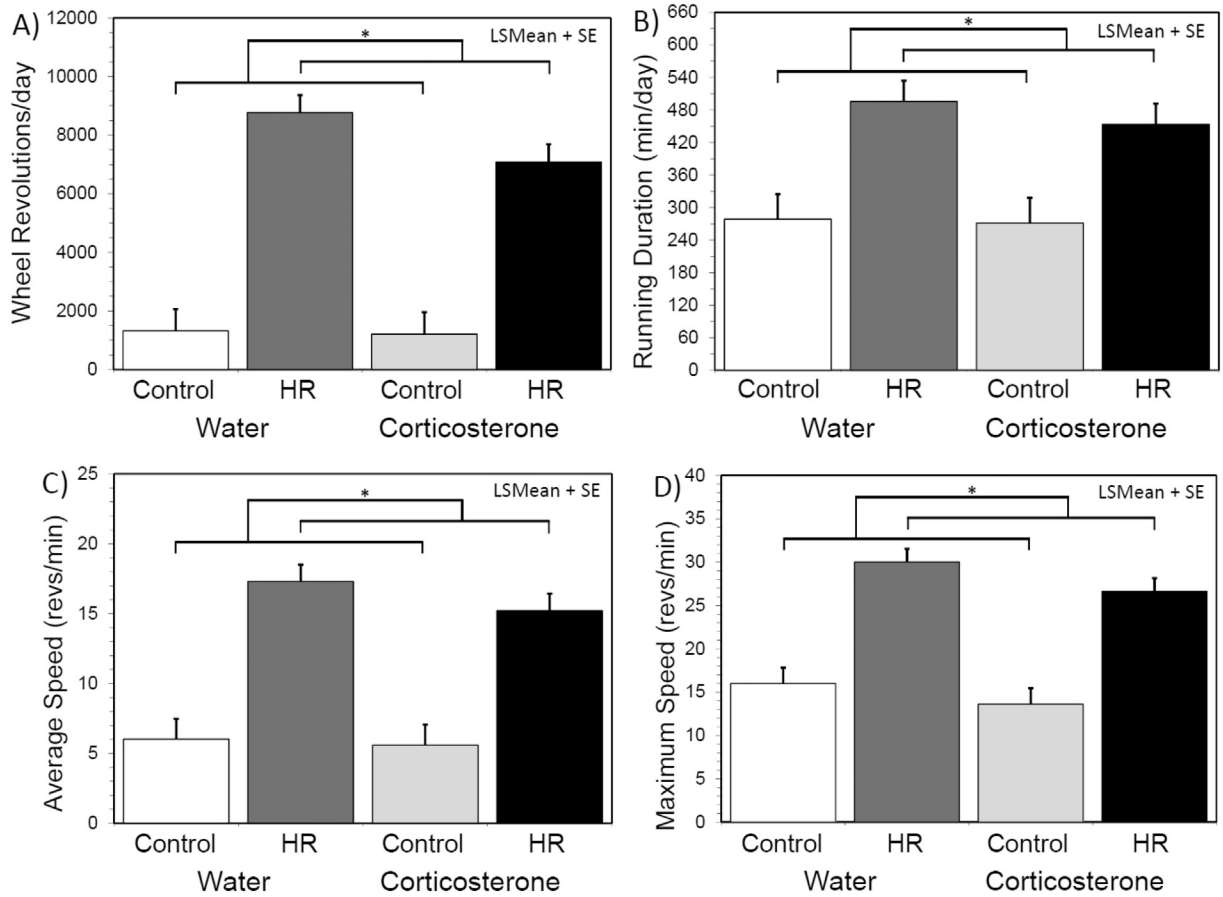

Fig. 4. Least squares means and standard errors for HR and C lines, with CORT solution or tap water treatment for (Table 1): (A) wheel running on days 5 and 6, measured as average total revolutions per day; (B) time spent running on days 5 and 6, measured as average 1-min intervals with at least one revolution; (C) average speed on days 5 and 6, measured as total revolutions/intervals; and (D) average maximum speed on days 5 and 6, measured as the single highest 1-min per day. Mice from HR lines ran a greater distance per day, for more minutes per day, and at higher average and maximum running speeds. * indicates $P < 0.05$.

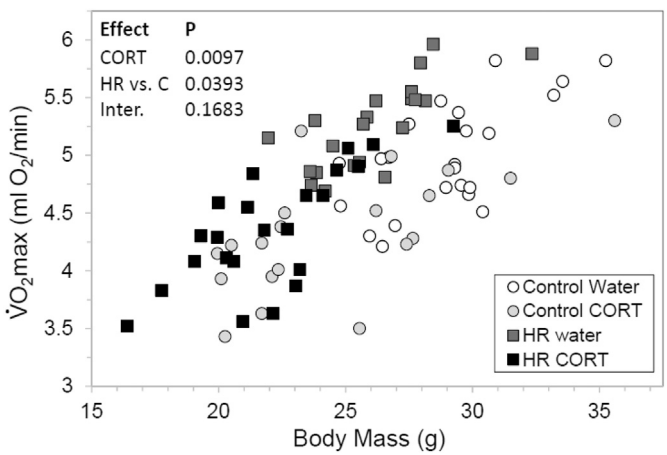

Fig. 5. Scatterplot of VO_2max values for HR and C mice with or without CORT solution treatment. With body mass as a covariate (Table 1), corticosterone treatment significantly reduced VO_2max for both HR and C mice ($P = 0.0097$), and HR mice had higher VO_2max than those from non-selected C lines ($P = 0.0393$).

VO_2max; therefore, consideration of how these traits responded to CORT administration may provide clues as to the mechanisms underlying reduced VO_2max. Humans with Cushing's syndrome (the main feature of which is hypercortisolism) have elevated hematocrit [79], which is consistent with our finding of elevated hematocrit in CORT-treated HR and C mice. Hematocrit is generally a positive predictor of VO_2max [53,80], but we observed increases in hematocrit with CORT treatment, whereas VO_2max decreased. These results suggest that hematocrit was not limiting to VO_2max in the present study, which is at odds with a previous study that found increases in hematocrit caused by the administration of an erythropoietin analogue to increase VO_2max in both HR and C mice [53].

Heart ventricle mass did not show an overall effect of CORT, but it tended ($P = 0.0847$) to be higher in HR mice ([53,57],as reported previously: [71]) and the CORT*linetype interaction was marginally non-significant ($P = 0.1014$). Mini-muscle mice also had larger hearts ($P = 0.0319$), as previously reported [69,71,72]. Though heart mass was not a predictor of VO_2max in a model including CORT treatment and linetype, a secondary (better-fitting) model including two-way and three-way interactions showed a significant interaction between heart mass, linetype, and CORT treatment. These results suggest that heart mass may be a limiting factor within some of the four experimental groups but not others.

Triceps surae muscle mass was significantly decreased by CORT treatment (although less so in mini-muscle individuals: Online Supplemental Table 2), suggesting that mitochondrial uptake of oxygen by skeletal muscle may have been at least partly limiting to VO_2max. Adding muscle mass and the various interaction terms described above to the model for analyzing VO_2max did not improve fit. These results are consistent with previous analyses of muscular hypertrophy in birds in response to CORT treatment [19,29].

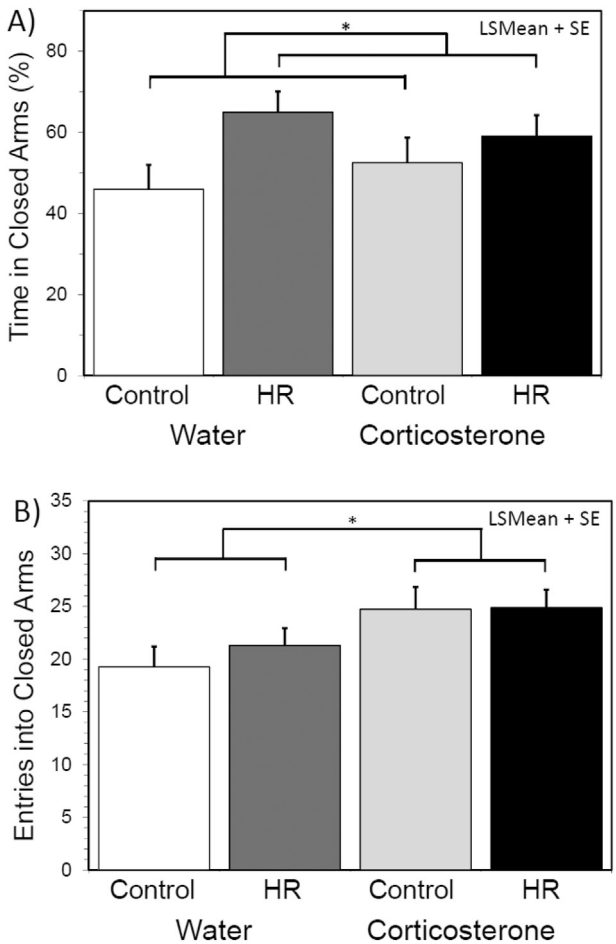

Fig. 6. Least squares means and standard errors for HR and C lines, with CORT solution or tap water treatment, for (A) percent time spent in closed arms of elevated plus maze during a 5-min test and (B) entries into closed arms of elevated plus maze. Mice from High Runner lines spent significantly more time in closed arms ($P < 0.05$ based on analyses of transformed values, Table 1), indicating increased anxiety-like behavior relative to mice from the non-selected C lines. Mice receiving CORT entered closed arms significantly more than those receiving tap water ($P < 0.05$, Table 1), indicating increased anxiety-like behavior as a result of treatment. Figure represents LSMs and SEs from analysis of untransformed values, whereas for statistical comparisons values were transformed as indicated in Table 1. * indicates $P < 0.05$.

4.3. Activity in home cages and in the elevated plus maze

Like wheel-running behavior, activity in home cages decreased as a result of CORT treatment, but once mice received access to wheels, home-cage activity was greatly reduced and the CORT effect disappeared. Our results for home-cage activity of mice housed without wheels concur with previous findings during administration of CORT to mice in drinking water [40]. HR mice typically exhibit higher home-cage activity than C mice when wheels are absent [52,57,58,81], though in our experiment this difference was not statistically significant. And, as in previous studies with these mice, we observed no difference in home-cage activity between HR and C mice when wheels were present (Table 1).

Although the elevated plus maze involves measurement of locomotor behavior, it is traditionally used to assess fear or anxiety in rodents and is not generally related to habituated activity in the home cage, nor to voluntary wheel running [82–84]. A previous study in rats found decreased entry into open arms of an EPM in response to exogenous CORT [85], which is consistent with our finding of increased entries into closed arms, suggesting increased anxiety. Long-term glucocorticoid treatment in water is known to induce an anxiety-like phenotype in mice [86], though corticosterone injections seems to lack a similar effect [87].

A previous study of male and female mice from generation 45 of this experiment compared two of the HR lines with one C line and reported that mice from both HR lines had a significantly greater percentage of arm entries into closed arms as compared with the C line; in addition, HR mice spent a greater percentage of time in the closed arms, with the difference being statistically significant for one of the two HR lines [61]. In the present study, we also found that male HR mice spent more time in closed arms (Fig. 6B), thus again suggesting that HR mice have increased anxiety-related behaviors as compared with mice from non-selected C lines. As mentioned previously, HR mice have circulating corticosterone levels ~2× those of C mice; this characteristic of the HR lines may result in the observed differences in anxiety-related behavior. This hypothesis could be tested with future studies that attempt to reduce the circulating CORT levels of HR mice, e.g., via adrenalectomy or with pharmaceutical agents. Interestingly, mice from C lines that received CORT had an increase in the latency to enter open arms, suggesting increased anxiety, whereas HR mice did not show this effect, perhaps because of a physiological ceiling effect related to their inherently higher circulating CORT concentrations.

The impacts of corticosterone on locomotor behavior may also extend between generations; multiple studies show that rats experiencing a high-glucocorticoid prenatal environment exhibit behavioral effects later in life. For example, female rats exposed to a stressor regimen for the last third of pregnancy (resulting in increased CORT levels) produced male offspring with reduced home-cage activity, an effect that did not occur for female offspring [88]. However, that study did not find effects of prenatal stress on total distance moved, distance moved in open arms, or entries into open arms in the EPM. (Acute exposure to a stressor before the EPM test did result in some interactive effects of prenatal stress.)

Rats that received dexamethasone during the last third of their gestational development were less likely to enter the open arms of an EPM as compared to controls, and significantly less likely to move into any arms [89].

4.4. Growth rate, body mass, and organ masses

Corticosterone treatment reduced adult body mass (Table 1, Fig. 2B). Mice did not differ in mass at weaning (1 day prior to beginning treatment), so the resulting body mass differences are the result of a significantly decreased growth rate caused by CORT. Such effects have been found many times previously for laboratory rodents [35,38,40,90], and we have previously reported that natural variation in plasma CORT correlates negatively with growth rate in females from both HR and C lines, suggesting that elevated CORT levels may be at least partly responsible for the reduced adult body sizes of HR mice [25,26].

Both HR and C mice of both sexes typically lose a substantial amount of fat mass (measured by whole-body noninvasive MRI) during the 6-day wheel-running trial used to choose breeders [70,91]. However, changes in total body mass are smaller because lean mass typically increases during this period. Although reproductive fat pads were smaller in HR mice with normal muscles than in C mice, mini-muscle mice had larger reproductive and subdermal fat pads (Table 1), as in a previous study [70].

In the present study, mice with supplemental CORT treatment lost more body mass during the 6-day period of wheel access, on both an absolute and percentage basis (Fig. 2C). Most of this effect may reflect loss of body fat, given that CORT treatment increased the mass of both reproductive and subdermal fat pads (adjusted for variation in body mass: Table 1). We are not aware of this effect being examined previously, but our results suggest that homeostatic mechanisms for body weight regulation [91] may have been disrupted by CORT

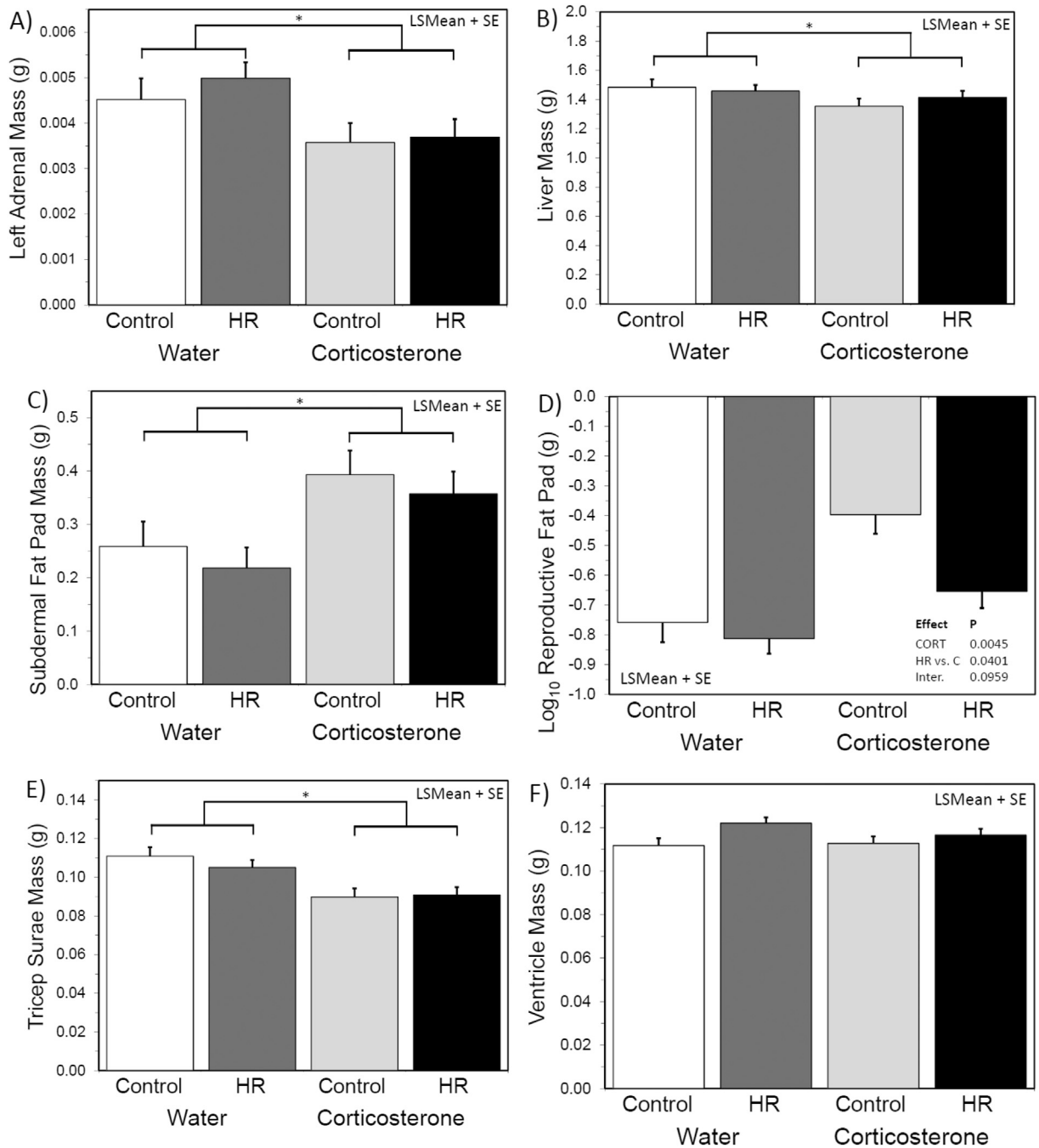

Fig. 7. Least squares means and standard errors (with body mass as a covariate) for HR and C lines, with CORT solution or tap water treatment, for body-size adjusted (analysis of covariance) masses of (a) left adrenal, (B) liver, (C) subdermal fat pad, (D) reproductive fat pad, (E) mean triceps surae, and (F) heart ventricle (g). Corticosterone treatment increased subdermal and reproductive fat pad mass, but decreased adrenal mass, liver mass, and triceps surae mass. In addition, for reproductive fat pad mass, CORT treatment had an interactive effect with linetype, causing a greater increase in Control lines than in HR lines (interaction P = 0.0959), and HR mice had smaller reproductive fat pad masses than those from non-selected C lines ($P = 0.0401$). [Note that for triceps surae mass the CORT*mini-muscle interaction was significant ($P = 0.0782$: Online Supplemental Table 2), but values show here are from a simplified model that did not include this term.] For other organ masses, see Results and Table 1. For liver, the figure represents LSMs and SEs from analysis of untransformed values, whereas for statistical comparisons values were transformed as indicated in Table 1. * indicates $P < 0.05$.

administration [92–94]. In addition, mice from C lines that received supplemental CORT experienced a redistribution of fat from subdermal to reproductive fat pads. In a similar study, rats receiving CORT in drinking water for 28 days showed increased visceral fat as compared with subdermal fat pad areas [35]. Moreover, human beings exhibiting hypercortisolism typically experience a redistribution of body fat from peripheral to central body areas [95]. Interestingly, mice from HR lines did not have a redistribution of fat between pads (Table 1), perhaps because their inherently higher baseline CORT levels protect them from this effect.

With body mass as a covariate, CORT treatment decreased adult tail length and liver mass. Corticosterone receptors in the liver trigger gluconeogenesis, hence increased plasma CORT may have resulted in increased mobilization of glucose from the liver and consequently reduced liver mass [96]. Decreased tail length may be related to the above-mentioned decrease in growth rate; though tail length is typically

allometrically related to body size [97], tail length can be altered in response to environmental conditions, such as temperature [98], and CORT treatment can reduce growth of long bones in mice [99]. Tail length and liver mass are not typically significantly different between HR and C mice [72,73] and did not differ in our experiment, although mini-muscle mice had significantly larger livers, as previously reported [69,70,72,73].

Corticosterone did not significantly impact kidney or testes mass. HR mice have been reported to have larger testes mass than C mice in some studies [72,100] but not others [72]. HR and C mice did not generally differ in relative kidney mass, but mini-muscle individuals had larger kidneys [69,72,73]. Previous studies have also reported larger kidneys in HR than C mice [69,72], although not always [73].

The decrease in adrenal mass caused by CORT administration may also have been associated with a decrease in thymus and/or spleen mass, although these organs were not weighed in the present study. Increased circulating glucocorticoids, resulting from either increased stress or direct supplementation, frequently induce a decrease in immune function, often expressed by decreased mass of thymus or spleen [101–105]. However, *evolutionary* increases in circulating glucocorticoid levels are not necessarily associated with suppressed immune function, as evidenced by comparative studies of population differences [105], including the High Runner versus Control lines in the present study [50]. Instead, evolved increases in glucocorticoids may be accompanied by "countermeasures" that reduce or eliminate potential deleterious effects [25].

4.5. Food and water consumption

Administration of CORT significantly increased food consumption as measured prior to wheel access. Water consumption was also significantly increased by CORT treatment, both before and during wheel access. The increased water consumption could simply reflect the fact that more water is required to process and digest more mouse chow. Alternatively, or in addition, CORT consumption can be reinforcing for oral self-administration [106].

4.6. Synthesis and conclusions

In considering results of the present study, the complexity of the glucocorticoid regulatory system and the many systems, organs, and tissues that interact with this endocrine axis is evident. As noted above, mice from the High Runner lines exhibit many physiological differences from those in the non-selected control lines, involving both motivation and ability for aerobically supported exercise ([11,25,57],e.g. [72,107]), and including elevated circulating CORT levels. The elevated CORT levels may have evolved in concert with other aspects of the hypothalamic-pituitary-adrenal (HPA) axis, such as receptor density in various target tissues [108], which have not yet been studied in these mice. (We note that adrenal gland mass has not been found to differ between HR and C mice [25]; this study,[72]). With regard to the brain, consistently elevated (both exogenous and stress-induced) CORT levels reduce CORT receptor densities in the amygdala and hippocampus [109], the latter of which is postulated to be involved in the regulation of locomotor behavior [49,110]; this may have been an underlying cause of the tendency for reduced wheel running and significantly reduced home-cage activity that we observed. More generally, voluntary wheel running is a motivated behavior, and HR mice show evidence of altered motivation for wheel running [111,112], along with altered dopamine, serotonin, and endocannabinoid signaling [49,112–115], with all of these neurotransmitter systems likely interacting with the HPA axis [116–121]. Given that the HPA axis also affects locomotor abilities in various ways (e.g., via effects on muscle), we may presume that the effects of CORT supplementation on physical activity observed here can be traced to effects on physical abilities for exercise as well as motivational and reward systems [11,122].

Overall, we observed primarily "negative" – clearly or potentially deleterious – effects of CORT treatment. Growth rate, home-cage activity, VO_2max, and calf muscle mass all decreased, whereas food consumption, fat pad masses, and an indicator of anxiety-like behavior increased. Similar to our results, Karatsoreous et al. (2010) found that a higher CORT dosage in water (100 μg/mL) than used here increased adiposity and decreased home-cage activity of adult mice; 100 μg/mL was also sufficient to significantly increase epididymal fat pad mass, specifically, in male mice after 4 weeks of treatment [123]. Gasparini et al. (2016) reported a 40% increase in body fat mass in response to a dosage of 50 μg/mL CORT administration in drinking water (identical to this study). The effects of CORT treatment that we observed for fat distribution (peripheral/visceral), food consumption, adrenal mass, and growth rate parallel the effects of excessive glucocorticoids (Cushing's syndrome) in humans [95,124,125]. We are not aware of studies of the alterations in physical activity that may accompany human hypercortisolism, but physical performance ability is negatively associated with circulating cortisol levels in older men and women [126], and depression frequently involves both high circulating cortisol levels and low activity levels [127–130]. Therefore, in a human clinical setting, it may be prudent to monitor physical activity when patients undergo extended periods of glucocorticoid treatment. If activity levels are indeed suppressed, then it could be appropriate to recommend physical exercise as an adjuvant.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.physbeh.2018.10.001.

Conflict of interest statement

The authors declare no conflict of interest.

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