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Plasma corticosterone response to acute and chronic voluntary exercise in female house mice

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Girard I., and T. Garland, Jr. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J Appl Physiol* 92: 1553–1561, 2002. First published December 7, 2001; 10.1152/japplphysiol.00465.2001.—Plasma levels of corticosterone (B) respond acutely to exercise in all mammals that have been studied, but the literature contains conflicting reports regarding how chronic activity alters this response. We measured acute and chronic effects of voluntary activity on B in a novel animal model, mice selectively bred for high voluntary wheel running. Female mice were housed with or without wheels for 8 wk beginning at 26 days of age. Wheel-access selection mice had significantly higher B at *night 8*, *day 15*, and *night 29*, compared with wheel-access controls. Elevation of B was an acute effect of voluntary exercise. When adjusted for running in the previous 20 min, no difference between wheel-access selection and control animals remained. No training effect on B response was observed. These results are among the strongest evidence that, in some animals, the acute B response is unaffected by chronic voluntary exercise. In mice without wheels, selection mice had significantly higher B than controls at *day 15*, *night 29*, and *night 50*, suggesting that selection resulted in a modulation of the hypothalamic-pituitary-adrenal axis. Growth over the first 4 wk of treatment was significantly and inversely related to average night B levels within each of the four treatment groups.

artificial selection; endurance training; evolution; glucocorticoids; growth; stress response

GLUCOCORTICOIDS PLAY AN IMPORTANT ROLE in the mobilization of energy reserves during physical activity by stimulating gluconeogenesis, promoting lipolysis of blood lipids, and increasing protein catabolism. A wide range of studies, in several species of mammals, have reported acute increases in the plasma concentration of glucocorticoids in response to submaximal (12, 30, 53), maximal (17, 32), and supramaximal (41, 43) exercise. Although the glucocorticoid response to light physical activity is variable, these previous studies have generally established that plasma glucocorticoid levels rise with moderate to exhaustive exercise and that higher exercise workloads elicit greater glucocorticoid response (16, 51).

The effect of chronic exercise on the glucocorticoid response to exercise is less clear (29). In studies of both humans and other mammals, researchers have reported increases (50, 52), decreases (53), and no change (6, 14) in the glucocorticoid response after training. Because forced-exercise training regimens are commonly employed to study endocrine responses in nonhuman animals (see Ref. 6), a confounding of the effects of exercise and psychological stress may contribute to the observed variation in training response. Chronic exposure to psychological stressors (novel environment or foot shock) failed to attenuate (42) or actually increased (22, 34) the glucocorticoid stress response in rats and mice, demonstrating that even lengthy habituation protocols may not adequately remove the psychological component of the stress response to forced exercise. Despite conflicting results, overall these studies suggest that physical training can affect the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis and that the effects of training vary with exercise type, intensity, and duration (51).

In addition to supporting activity via metabolic modulation, glucocorticoids also have complex effects in the central nervous system (54), including effects on locomotor behavior (2, 7, 11). For example, in captive screech owls, peaks in corticosterone corresponded with periods of high locomotor activity at the time of dispersal (3). In red-eared slider turtles (*Trachemys scripta elegans*), administration of exogenous corticosterone in physiological doses increased locomotor activity over the next 48 h (10). In rats, corticosterone was necessary for the occurrence of schedule-induced wheel running after adrenalectomy (33), and chronic corticosterone administration to intact animals resulted in increased locomotor activity (56). Corticosterone is also self-administered intravenously by catheterized rats (37) and thus is thought to have reinforcing properties. The self-reinforcing property may relate to the expression of "sensation-seeking behavior" (37) and perhaps also to the motivation for voluntary wheel running.

We have developed a novel mouse model to study the evolution of physiological traits that support increased levels of locomotor activity, including the role of glucocorticoids in voluntary exercise. After 24 generations of artificial selection for high voluntary wheel running, selection-line mice ran an average 2.7 times more rev-

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olutions per day compared with mice from nonselected control lines (26). Daily wheel-running distances among selection-line females averaged 16.2 km at *generation 24* (21). Females of both selection and control lines run enough to train (physically condition) themselves, as demonstrated by the increased activity of mitochondrial (e.g., citrate synthase, cytochrome-*c* oxidase, pyruvate dehydrogenase) and glycolytic enzymes (e.g., hexokinase) (24), and increased hematocrit and blood hemoglobin concentration (unpublished data) after 8 wk of wheel access. Selection-line females also have reduced body mass (48) and reduced fat mass (45) compared with control lines, suggesting a possible trade-off between energy expenditures for growth and activity (5).

Because wheel running is both energetically expensive and potentially physiologically taxing, we expected that corticosterone played a role in supporting this type of voluntary exercise. We developed four specific hypotheses. First, as has been shown in many previous studies of mammals, we predicted that plasma corticosterone levels would rise acutely in proportion to the amount of wheel-running activity during some relatively short period of time before sampling. Second, because mice from selection lines exhibit much higher activity on running wheels (at night), we predicted that, compared with mice from control lines, they would show higher levels of corticosterone during activity. Third, we predicted that the acute response to wheel running would increase over time with chronic wheel access. Voluntary exercise is presumably less psychologically stressful than forced running, such that the corticosterone response we measured should predominately reflect exercise-induced changes. In voluntarily exercising female hamsters, the model most similar to our own, chronic exercise led to enhanced glucocorticoid synthesis and release (6). Fourth, we hypothesized that, in the absence of wheels, corticosterone levels in mice from selection lines would not differ from those in control-line mice. These hypotheses were tested in selection and control lines of female mice housed either with or without wheel access for 8 wk beginning at 26 days of age.

METHODS

Study animals. Juvenile female Hsd:ICR mice (*Mus domesticus*) were used for the experiment, which was approved by the Institutional Animal Care and Use Committee (IACUC). Females were chosen as the study sex because they demonstrate greater levels of voluntary wheel running than males and have higher peak corticosterone levels in response to exercise (12). Female mice were taken from *generation 21* (second litters) of an artificial selection experiment for high voluntary wheel running (46). At *generation 21*, first-litter females from the four replicate selection lines were running an average of 14,300 revolutions/day on *days 5* and *6* of a 6-day test, whereas females from the four randomly bred control lines averaged 5,100 revolutions/day. Pups for this study were weaned at 21 days of age, at which time two females from each of six families per line ($n = 96$ mice) were chosen at random. Each female was weighed, toe clipped for identification, and housed individually. At 25–27 days of age, the first blood sample (*day 0*) was collected (see *Blood sampling for corticosterone*). Immediately after blood sampling, one sister of each pair (no-wheel group) was individually housed in a standard shoebox cage ($27 \times 17 \times 12.5$ cm), and the other (wheel-access group) was individually housed in a similar cage but with continuous access to a large running wheel (1.12 m circumference) through a short tube. Animals were maintained on a 12:12-h light-dark cycle with ad libitum access to food (Harlan Teklad Rodent Diet W8604) and water and were weighed weekly. Activity in the wheels was automatically recorded in 1-min bins by computer. Wheel-running data were summarized as total revolutions per day, total number of active 1-min intervals (bins in which any revolutions were recorded) per day, and mean speed (total revolutions divided by number of active intervals). Stage of estrous cycle was determined by vaginal smears within 2 h after blood sampling, beginning at 5 wk of age, following the five stages outlined by Rugh (40). Treatment continued for 52 days.

Blood sampling for corticosterone. Blood samples for corticosterone determination were collected at six times over 52 days with 7–14 days between sampling. Blood was collected 1–3 h after lights on (*days 0*, *15*, *43*) and 1–3 h after lights off (*nights 8*, *29*, *50*). Small volume (<50 μl) blood samples were drawn by retro-orbital puncture without anesthesia in <2.0 min from first disturbing the mouse. Blood sampling was performed by an experienced researcher (I. Girard) under a protocol approved by the IACUC. Mice were monitored by the staff veterinarian, and none of the 96 females suffered any detectable injuries as a result of the six blood collections.

Blood was collected in heparinized microhematocrit tubes, centrifuged, and the separated plasma was stored at −80°C until analysis. Steroids were extracted and corticosterone was purified as in a previous experiment (12) (for a complete methodology, see Ref. 1). In brief, steroids were extracted from plasma with 7.5 ml ether in two washes. The corticosterone fraction was eluted with 50% ethyl acetate in isooctane from a celite microcolumn with a 1:1 ethylene glycol-to-propylene glycol solid phase. Corticosterone concentrations (ng/ml plasma) were determined for duplicate aliquots by radioimmunoassay. Specific binding in the corticosterone assays was 48.1 ± 4.9% (SD, $n = 11$) and the limit of detection was 2 ng/ml; intra-assay coefficient of variation was <5.5%; interassay coefficient of variation was 11.1% for the low-corticosterone standard and 9.2% for the high-corticosterone standard. A maximum of 50 samples (in duplicate) could be included in one assay run. As our primary interest was a comparison between selection and control animals within a treatment, samples within a treatment group were analyzed together in a single run to reduce interassay variation.

Statistical analyses. Statistical analyses were performed by using PROC MIXED in SAS (6.0). For all analyses, the effect of selection history was included as a fixed main effect, and replicate lines were nested within selection history as random effects. The effects of selection history and wheel access on body mass each week was tested with a two-way mixed-model ANOVA. The effect of selection history on wheel running was tested with a mixed-model analysis of covariance (ANCOVA) including wheel freeness as a covariate.

Statistical analyses of corticosterone were conducted separately for each treatment group, except for *day 0* before treatment began. Statistical comparisons between treatments were not made, as our hypotheses concerned the effects of selection history on corticosterone. The effect of selection history on corticosterone was tested for each treatment with a mixed-model ANCOVA, including time required to collect blood from first disturbance of the animal, time of

day, (z-transform of time of day)^2, arousal state (sleeping/resting or alert/active), wheel freeness, and stage of estrous cycle (where applicable) as covariates or cofactors. In wheel-access mice, the acute effect of wheel running on corticosterone was tested by using revolutions in the 20 min before sampling as a covariate in the above model, replacing arousal state. In preliminary analyses, wheel running in the previous 20 min was the best predictor of corticosterone (highest R^2) among the measures of wheel activity tested (revolutions in the previous 5, 10, 20, 30, 60, and 120 min).

The chronic effect of wheel running on the corticosterone response to exercise was examined in wheel-access mice by testing for both a main effect of treatment day (i.e., sample *nights 8*, *29*, and *50*) and a change in the acute effect of wheel running over time (interaction of wheel running and treatment day). The significance of the main effect of treatment and of the interaction (wheel running in previous 20 min × treatment day) was tested in a repeated-measures, mixed-model ANCOVA with covariates and cofactors as described above for the acute-effect model.

The relationship between corticosterone and body mass increase over the rapid growth phase (*days 0-29*) was examined with a two-way mixed-model ANCOVA with selection history and treatment as main effects and body mass at *day 29* as the dependent variable. The model included mass at *day 0*, mean night corticosterone (average of *nights 8* and *29*), and mean day corticosterone (average of *days 0* and *15*) as covariates, as well as the interactions between each of the two corticosterone variables and selection history. Further analyses on the effects of mass at *day 0* and mean night corticosterone on mass at *day 29* were conducted on each of the four treatment groups separately.

Although the work of Borer (6) suggests that acute response of plasma corticosterone levels should increase with training (exposure to running wheels), we did not have clear predictions for changes in basal (daytime) levels or active (nighttime) levels in animals without wheel access. Therefore, for simplicity, we report two-tailed P values throughout.

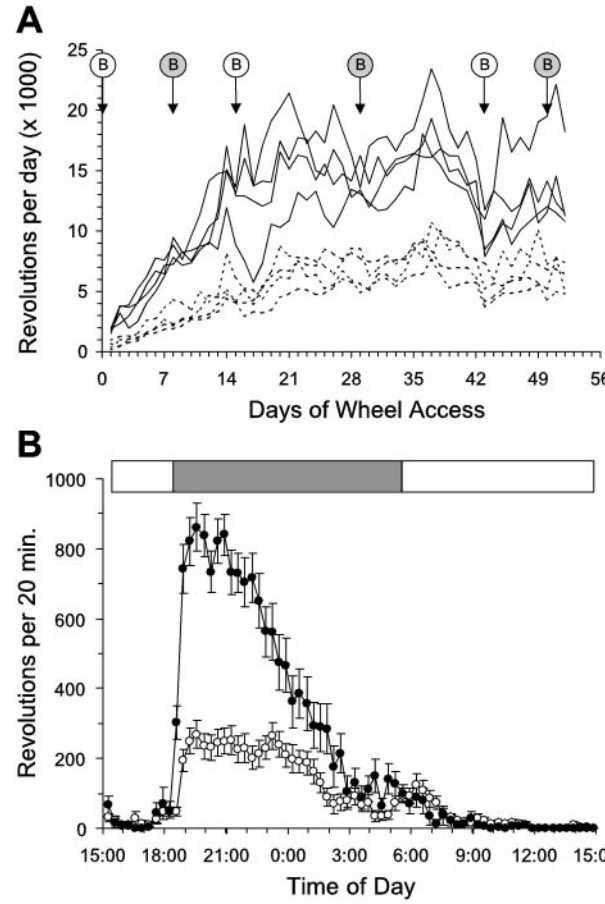

Fig. 1. Wheel running in selection and control lines of female mice. *A*: mean revolutions per day for each of four replicate selection (solid lines) and control (broken lines) lines over 52 days of wheel access with the occurrence of daytime (open icons) and nighttime (shaded icons) blood sampling for corticosterone. *B*: example of the circadian pattern of wheel running in selection (●) and control (○) females over 24 h during *week 4* of treatment, 3 days before blood sampling. Values are means ± SE.

RESULTS

Wheel running. On average, mice from selection lines ran more revolutions per day than those from control lines during each of the 52 treatment days (Fig. 1*A*). Wheel running generally increased over the first 3 wk of wheel access, then stabilized over *weeks 4–8*. Over *days 14-52*, mice from selection lines ran an average of 14,474 ± 741 (SE) revolutions/day, and mice from control lines ran an average of 6,456 ± 426 revolutions/day [effect of selection history: $F(1, 6) = 23.3$, $P = 0.003$]. Most of the difference in number of wheel revolutions between selection and control lines was caused by the greater average running speed [$F(1, 6) = 31.3$, $P = 0.002$] of the selection lines (30.3 ± 1.21 revolutions per active 1-min interval) compared with the control lines (14.1 ± 0.67 revolutions/min), with no statistically significant difference in the average time spent running per day over *days 14-52* [selection lines: 474 ± 14.5 min/day; control lines: 423 ± 21.9 min/day; $F(1, 6) = 0.84$, $P = 0.30$].

The circadian pattern of wheel running appeared to be similar in control and selection females (Fig. 1*B*). Mice usually began running at lights off, with the peak running period occurring 1–4 h after lights off. Over the 3 h of peak activity, selection animals accrued 166.6 ± 3.36 active 1-min intervals and control animals accrued 131.5 ± 11.2 active 1-min intervals (averaged over *days 14-52*). Mean wheel-running speeds averaged 42.5 ± 1.76 revolutions/min in selection mice and 16.0 ± 0.78 revolutions/min in control mice over *days 14–52*. Activity during the 3-h peak period accounted for 48.9% of total daily revolutions and 35.0% of total active intervals in selection animals, and 32.6% of total daily revolutions and 31.0% of total active intervals in control animals. Sampling times for corticosterone during nocturnal periods thus corresponded with periods of peak activity. A second, brief period of less intense activity coincided with lights on, after which wheel-running activity was negligible through the rest of the illuminated period.

Females monitored daily for estrous cycling during *week 6* of treatment demonstrated increased running on the night of proestrous compared with diestrous and postestrous [repeated measurements over *days 41–50*: $F(3, 405) = 16.9$, $P < 0.001$]. On average, females ran 22% more revolutions during proestrous than during

diestrous and postestrous, with no significant difference in the effect of estrous phase on selection and control females [interaction of estrous × selection history: $F(3, 6) = 1.01$, $P > 0.4$].

Plasma corticosterone levels in day and night samples. The effects of selection history, arousal state, and stage of estrous cycle on plasma corticosterone levels are summarized in Table 1. No differences in morning plasma corticosterone levels existed between female mice from selection and control lines just before initiation of treatment at 26 ± 1 days of age. In wheel-access mice, females from selection lines had significantly higher plasma corticosterone levels at *night 8*, *day 15*, and *night 29*. In mice housed without wheels, selection-line females had significantly higher plasma corticosterone levels at *day 15*, *night 29*, and *night 50*. Arousal state (resting/sleeping or alert/active) was a highly significant predictor of corticosterone level in most samplings of wheel-access mice (*night 8*, *day 15*, *night 29*, and *day 43*) but was a significant cofactor only for *day 43* in no-wheel mice. The number of alert/active animals at the first morning sampling was 4 selection and 10 control mice (wheel-treatment groups combined, total $n = 96$). In wheel-access mice, the number of alert/active animals was as follows: *night 8* (17 selection, 19 control); *day 15* (8 selection, 11 control); *night 29* (24 selection, 21 control); *day 43* (8 selection, 13 control); *night 50* (24 selection, 23 control). In no-wheel mice, the number of alert/active animals was as follows: *night 8* (11 selection, 9 control); *day 15* (5 selection, 11 control); *night 29* (17 selection, 15 control); *day 43* (14 selection, 10 control); *night 50* (22 selection, 20 control).

When stage of estrous cycle was a significant predictor of corticosterone (*day 15* and *night 29* in no-wheel animals only), females in proestrous and early estrous had higher corticosterone levels than those in diestrous or postestrous. Time of day had a significant, positive effect on corticosterone levels at *day 0* [combined treatment groups: $F(1, 20) = 8.12$, $P = 0.01$], *night 8* [no wheel: $F(1, 30) = 5.79$, $P = 0.02$], *day 15* [wheel access: $F(1, 30) = 4.95$, $P = 0.03$; no wheel: $F(1, 6) = 5.75$, $P = 0.02$], and *night 29* [no wheel: $F(1, 30) = 6.34$, $P = 0.02$]. However, the (z-transformed time of day)^2 variable was not significant in any of the above analyses. The time required to draw the blood sample (grand mean = 61.1 ± 1.9 s, range = 31–102 s) was a significant (positive) covariate only in a single sample group [*day 15* no wheel: $F(1, 32) = 11.4$, $P = 0.002$].

Acute and chronic effects of wheel running on corticosterone. Although differences in the overall plasma corticosterone levels were found (see above), once plasma corticosterone levels were adjusted statistically for the amount of wheel running 20-min immediately before sampling, no selection-history differences remained [$F(1, 6) < 4.2$, $P > 0.08$; Table 3].

At night, females from selection lines tended to run more revolutions in the 20 min before blood sampling than did control-line mice, but the difference was significant only at *night 29* (Table 2). On *day 15*, control-line females ran significantly more than selection-line females in the 20 min before sampling, although the amount of wheel running was relatively low compared with night sampling periods. The number of revolutions in the 20 min before blood sampling was a significant positive predictor of plasma corticosterone levels at *day 15* and in all night samples (Table 3). The acute effect of wheel running was not different between control and selection animals in any sampling period [interaction of wheel-running × selection history: $F(1, 6) < 2.0$, $P > 0.3$], as illustrated in Fig. 2. The partial regression coefficients ($b' \pm$ SE) for the effect of wheel running on (square-root transformed) plasma corticosterone were 0.015 ± 0.006 (*night 8*), 0.012 ± 0.003 (*night 29*), and 0.019 ± 0.006 (*night 50*).

Chronic access to wheels did not affect the acute response of corticosterone to wheel running (Fig. 2). Repeated-measures analysis demonstrated neither a

Table 1. *Plasma corticosterone levels with and without access to running wheels*

	Selection Lines	Control Lines	$P_{\text{selection history}}$	P_{arousal}	P_{estrous}
		Combined			
Day 0	23.5 ± 8.42 (18)	16.5 ± 7.67 (14)	0.98	0.76	
		Wheel access			
Day 15	39.2 ± 7.76 (22)	38.4 ± 13.4 (21)	0.033	0.002	0.84
Day 43	66.5 ± 15.8 (20)	82.9 ± 19.3 (21)	0.089	<0.001	0.13
Night 8	195.8 ± 30.3 (20)	119.4 ± 19.4 (22)	0.039	0.001	
Night 29	315.9 ± 32.6 (20)	198.8 ± 21.3 (24)	0.039	0.016	0.90
Night 50	218.1 ± 23.6 (23)	189.8 ± 24.7 (22)	0.39	0.87	0.18
		No wheel			
Day 15	47.3 ± 8.87 (23)	20.5 ± 6.89 (22)	0.004	0.95	<0.001
Day 43	61.8 ± 15.2 (22)	34.0 ± 10.8 (22)	0.86	0.002	0.64
Night 8	140.7 ± 20.8 (23)	137.7 ± 29.0 (19)	0.49	0.46	
Night 29	276.0 ± 40.0 (21)	144.9 ± 18.3 (22)	0.011	0.89	0.001
Night 50	161.9 ± 21.1 (24)	81.3 ± 14.2 (24)	0.038	0.85	0.30

Values are means ± SE given in ng/ml plasma; n shown in parentheses for diurnal and nocturnal sampling periods. Significance of the effects of selection history, arousal state, and stage of estrous are from nested analysis of covariance (ANCOVA), including time required to draw blood sample, time of day, and (z-transformed time of day)^2 as cofactors or covariates. Treatment groups (wheel access or no wheel) were analyzed separately except at *day 0*. Treatment began when mice were 26 ± 1 days old; the first mice began estrous cycling at 40 days of age.

Table 2. *Voluntary wheel running in 20 min before blood sampling*

	Revolutions		$P_{\text{selection history}}$
	Selection lines	Control lines	
Day 15	40 ± 21	88 ± 25	0.04
Day 43	94 ± 46	119 ± 26	0.72
Night 8	180 ± 34	73 ± 33	0.06
Night 29	580 ± 42	217 ± 31	0.001
Night 50	481 ± 99	185 ± 99	0.07

Values are means ± SE given in revolutions per 20 min. Samples sizes as shown in Table 1 for wheel-access group.

significant effect of treatment day [$F(2, 77) = 1.04$, $P = 0.36$] nor a change in the acute effect of wheel running (revolutions in previous 20 min) on plasma corticosterone over time in the three night samples [revolutions × treatment day interaction: $F(2, 6) = 1.00$, $P = 0.37$]. As was demonstrated in the acute-effects analysis above, the acute effect of wheel running in the previous 20 min was a significant predictor [$F(1, 77) = 19.7$, $P < 0.0001$] of night corticosterone, but no other cofactors or covariates, including selection history [$F(1, 6) = 0.03$, $P = 0.87$], had significant effects in the repeated-measures analysis. Additional evidence for the lack of attenuation in the corticosterone response over time is seen in the comparison of *night 50* corticosterone values between groups. Selection females demonstrated no difference in corticosterone adjusted for acute wheel running compared with control females [selection history: $F(1, 6) = 1.17$, $P = 0.32$; history × revs: $F(1, 6) = 0.05$, $P = 0.82$; see Table 3], even as their cumulative running effort (650,814 ± 33,801 revolutions over *days 1–50*) was 2.3-fold greater than that of controls (281,739 ± 18,668).

Body mass. Body mass was not significantly different between control and selection lines either at weaning [$n = 96$, $F(1, 6) < 0.5$, $P > 0.8$] or just before initiation of treatment [$F(1, 6) < 0.5$, $P > 0.7$; Fig. 3]. Mass increased over the first 3 wk of treatment in all animals and was significantly higher in control females than in selection females in *week 4* [*day 22*: $F(1, 6) = 6.91$, $P = 0.039$], *week 5* [*day 29*: $F(1, 6) = 6.14$, $P = 0.048$], *week 7* [*day 43*: $F(1, 6) = 6.13$, $P = 0.047$], and *week 8* [*day 50*: $F(1, 6) = 7.03$, $P = 0.038$]. The main effect of wheel access was significant only at *day 50* [$F(1, 6) = 10.2$, $P = 0.019$], when animals housed without wheels had higher mass than wheel-access

Table 3. *Plasma corticosterone levels adjusted for amount of wheel running in 20 min before sampling*

	Adjusted Corticosterone		$P_{\text{wheel running}}$	$P_{\text{selection history}}$
	Selection lines	Control lines		
Day 15	43 (25, 60)	29 (12, 47)	0.003	0.09
Day 43	75 (37, 113)	60 (22, 96)	0.31	0.76
Night 8	138 (92, 193)	114 (76, 161)	0.02	0.51
Night 29	211 (144, 290)	243 (183, 311)	0.002	0.57
Night 50	176 (136, 216)	208 (169, 246)	0.007	0.32

Values are back-transformed least-square means with 95% confidence intervals (lower, upper) given in ng/ml plasma. Significance of the effects of wheel running and selection history are from nested ANCOVA, which also included wheel freeness, stage of estrous, time required to draw blood sample, time of day, and (z-transformed time of day)^2 as cofactors or covariates.

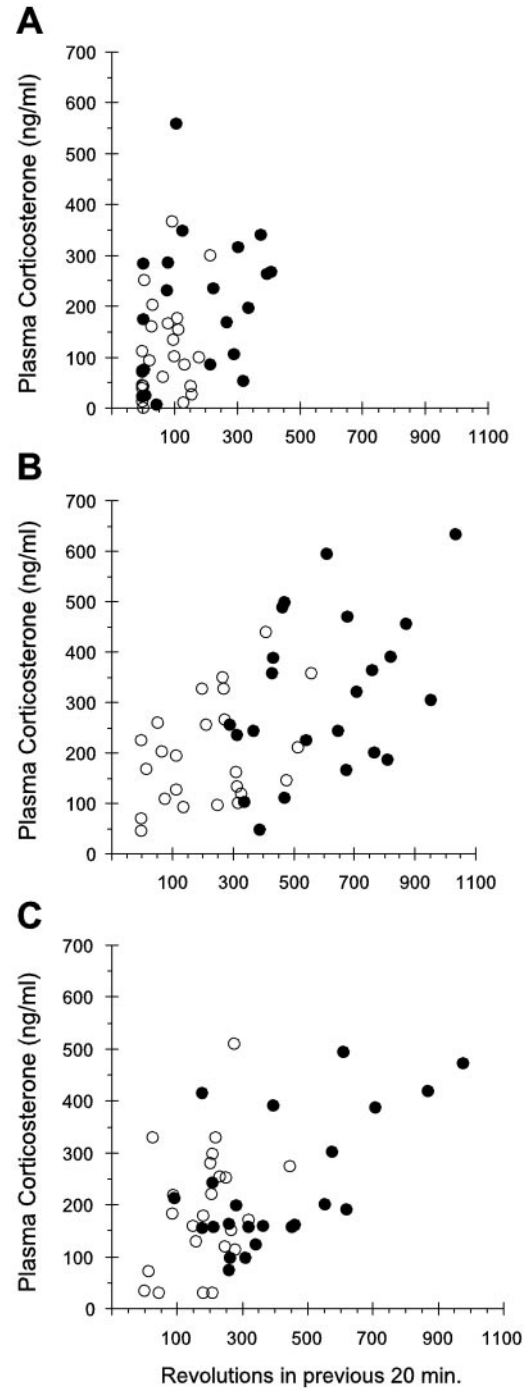

Fig. 2. Acute effect of wheel running on plasma corticosterone. Plasma corticosterone level (ng/ml) in selection (●) and control (○) females is significantly correlated with the number of wheel revolutions in the 20 min before blood sampling during the dark period on *night 8* (*A*), *night 29* (*B*), and *night 50* (*C*) of wheel access in nested analyses of covariance (ANCOVA), with time required to collect blood, time of day, (z-transformed time of day)^2, wheel freeness, and stage of estrous cycle (where applicable) as covariates or cofactors.

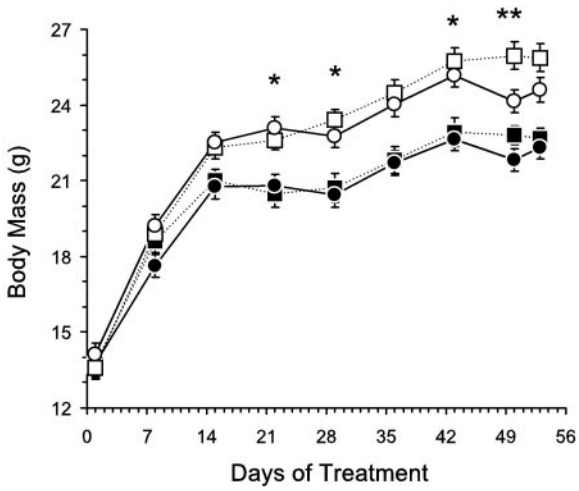

Fig. 3. Body mass in selection female mice with (●) and without (■) wheel access, and in control female mice with (○) and without (□) wheel access over 8 wk of treatment beginning at 26 ± 1 days of age. Values are means ± SE. *Significant effect of selection history [$F(1, 6) > 6.1, P < 0.05$]. **Significant effects of both selection history and treatment [$F(1, 6) = 10.2, P < 0.02$] in a two-way nested ANCOVA.

mice. No significant interaction between selection history and treatment existed in any week.

Relationships among wheel running, plasma corticosterone, and growth. Over the first 4 wk of treatment (*days 0* through *29*), selection females increased mass by an average of 6.83 ± 0.32 g (treatment groups combined), compared with an increase of 9.24 ± 0.32 g in control mice. Body-mass increase over the first 4 wk was inversely related to the average night corticosterone values in each of the four groups. In an analysis of all animals, mass at *day 29* of treatment was predicted by mass at *day 0* [partial $F(1, 63) = 23.2, P < 0.0001$, positive slope] and by mean night corticosterone [$F(1, 63) = 12.7, P < 0.0001$, negative slope] but not by mean day corticosterone [$F(1, 63) = 1.87, P = 0.18$, negative slope]. In this analysis, selection history [partial $F(1, 6) = 5.57, P = 0.06$], treatment [$F(1, 63) = 1.42, P < 0.24$], and the interactions of selection history and treatment with corticosterone [$F(1, 6) < 2.15, P > 0.15$] were not significant predictors of mass at *day 29*.

When each group was examined separately, mean night corticosterone was a negative and statistically significant predictor of mass at *day 29* in each, as illustrated in Fig. 4, *A* and *B* [significance of the effect of mean night corticosterone in wheel-access selection: partial $F(1, 14) = 4.85, P = 0.045$; wheel-access control: $F(1, 17) = 7.94, P = 0.012$; no-wheel selection: $F(1, 16) = 12.8, P = 0.003$; no-wheel control: $F(1, 13) = 10.6, P = 0.006$]. (Note: exclusion of the selection animal with a mean corticosterone value of 593 ng/ml did not change the results.) Interestingly, initial mass (*day 0*) was a significant positive predictor ($F > 10.6, P < 0.007$) of mass at *day 29* in three groups but was not a significant predictor in the analysis of wheel-access control animals [$F(1, 17) = 1.57, P = 0.23$, but slope was still positive].

In further analyses of wheel-access animals only, the effect of wheel running on growth in selection and control animals was examined for each group (Fig. 4*C*). In selection animals, only initial mass [partial $F(1, 13) = 12.8, P = 0.003$, positive slope] predicted mass at *day 29*, and neither cumulative running effort [sum of revolutions over *days 1-28*: $F(1, 13) = 0.81, P = 0.38$] nor mean night corticosterone [$F(1, 13) = 0.45, P = 0.51$] had a significant effect on mass when all three were included as covariates. In control animals, both

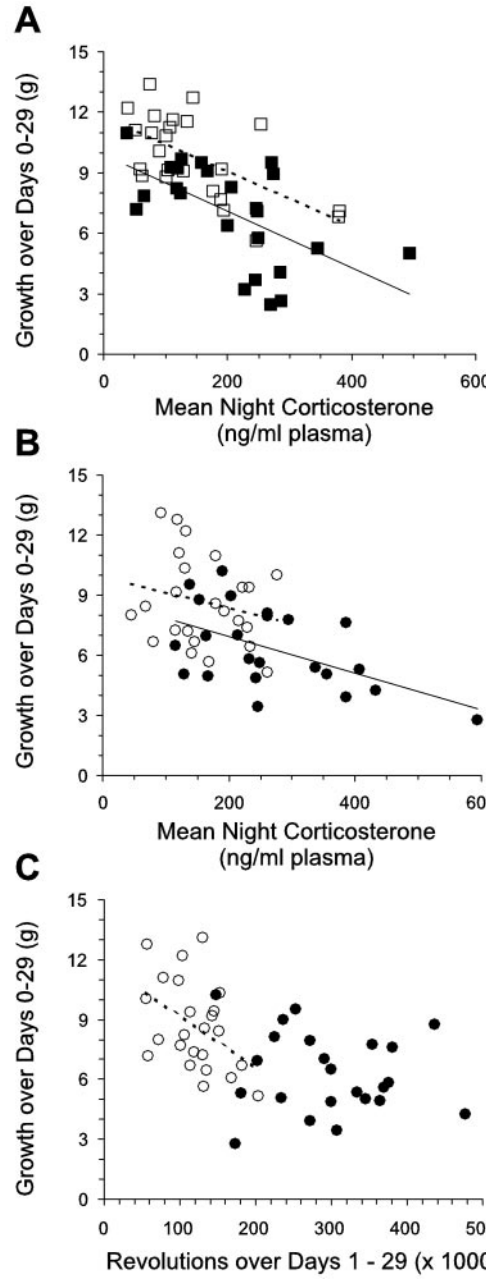

Fig. 4. Growth of female mice from selection (■, ●; solid lines) and control (□, ○; broken lines) lines over 29 days of treatment beginning at 26 ± 1 days of age is significantly and negatively correlated with the mean plasma corticosterone levels (ng/ml) measured on *nights 8* and *29* in no-wheel treatment (*A*) and wheel-access groups (*B*). Among wheel-access animals, cumulative running effort (sum of revolutions over *days 1-28*) was a significant additional predictor of mass at *day 29* in control animals but not in selection animals (*C*).

cumulative running effort [$F(1, 15) = 8.27$, $P = 0.011$] and mean night corticosterone [$F(1, 15) = 7.89$, $P = 0.013$] had significant negative effects on mass at *day 29*, but initial mass [$F(1, 15) = 2.35$, $P = 0.15$, positive slope] was not a significant predictor of mass at *day 29*. Multicollinearity was not a problem in the above analyses, as mean night corticosterone was not significantly correlated with cumulative running effort in either selection ($r < 0.01$) or control ($r = 0.11$) animals.

DISCUSSION

Our results demonstrate that the acute response of plasma corticosterone is directly and linearly related to the amount of voluntary exercise in house mice, confirming the general findings of a proportional plasma corticosterone response under conditions of moderate or heavy exercise. As demonstrated in earlier studies of chronic wheel access in these mice (21, 27, 48), females voluntarily exercised for several hours each night at levels that appeared to be of moderate to heavy intensity. Mean wheel-running speeds during peak activity over *weeks 3-8* were 32 (control) and 85% (selection) of the estimated maximal aerobic speed (3.4 km/h for a 23-g mouse; from Fig. 6 of Ref. 28). As expected, selection animals had higher absolute corticosterone levels than controls during two nocturnal sampling periods (*nights 29* and *50*), but this difference was explainable by the greater wheel running of selection mice in the minutes before sampling.

No evidence of a training effect on corticosterone response to exercise was found after chronic wheel access, as the acute response of corticosterone to the amount of wheel running in the previous 20 min was consistent across 50 days of wheel access and never differed between control and selection groups. These results appear to be among the first reports of corticosterone response to chronic high voluntary exercise in a nonhuman animal (see Ref. 6 for changes in basal steroidogenesis in voluntarily exercising hamsters) and may provide the clearest evidence that, in some animals, the acute corticosterone response is unaffected by chronic, moderate to heavy voluntary exercise.

Although no selection-history effect was found among wheel-access animals, selection females housed in regular cages without wheels had significantly higher corticosterone levels than controls without wheels in one daytime and two nighttime sampling periods. Because we have no measure of locomotor activity in groups housed without wheels, we cannot determine whether corticosterone is related to home-cage activity. In a separate study of home-cage activity at *generation 24*, selection females housed in photobeam activity-cages had 3.7-fold greater ambulations than control mice on the second day of a 2-day test in cages larger than those used in the present study (38). These data suggest that the differences in corticosterone levels among animals in home cages might also be explained in part by differences in locomotor activity in the absence of wheels. However, we do not know if such differences would persist for several weeks. Moreover, results from previous studies suggest that home-cage activity is physiologically less taxing than wheel running, which does elicit responses consistent with endurance-training effects. Compared with "sedentary" animals, wheel-access mice demonstrated increases in maximum aerobic capacity (47) and in metabolic capacities of the hindlimb (24) and endurance of the gastrocnemius (57). It is unlikely that selection mice without wheels were exercising at the same intensity as their counterparts with wheel access, even though the treatment groups demonstrated plasma corticosterone levels of similar magnitude. Therefore, home-cage activity may be a poor predictor of corticosterone levels in animals without wheels, a hypothesis that could be tested with the use of photobeam activity cages.

Alternatively, the high corticosterone levels of selection females housed without wheels may be reflective of a modulation of the HPA axis as a result of selection for increased wheel-running behavior. Upregulation of the HPA axis could result in both increased wheel-running behavior and increased corticosterone release downstream. One potential mediator of such a relationship is corticotropin-releasing factor (CRF). CRF stimulates corticotropin and glucocorticoid secretion but also has behavioral effects in the central nervous system (31, 36), independent of its action on the anterior pituitary. Central administration of CRF can produce dramatic increases in locomotor activity in both rats (25, 44) and mice (19), depending on dose and testing conditions. This effect may be independent of CRF stimulation of the pituitary (19), as locomotor activation was not blocked by dexamethasone at a dose that prevented a CRF-induced rise in plasma corticosterone (8, 9), nor by hypophysectomy (20). Also, corticosterone itself may also play a role in promoting locomotor behavior.

Another explanation for the unexpectedly high corticosterone levels in the selection animals housed without wheels can be proposed but is difficult to test. It is possible that, for animals selectively bred for high wheel-running activity, the absence of a wheel is psychologically stressful. However, selection females with locked wheels unable to rotate actually demonstrated lower frequencies of biting and sniffing behaviors compared with controls (27). The behavior of mice unable to run on wheels is therefore not characterized by the increase in stereotypes normally observed in rodents under stress (55).

An unexpected finding was the consistent negative correlation between growth and endogenous corticosterone levels measured on *nights 8* and *29* in each of the four treatment groups. We have previously reported that selection-line females demonstrated reduced body mass (*generation 14*; Ref. 48) and reduced fat mass (*generation 13*, whole body fat mass; Ref. 49; *generation 17*, retroperitoneal fat pad weight; Ref. 18) compared with control-line females. In the present study, lower early growth rates in selection females led to a significant difference in mass by the third week of treatment compared with controls, and this difference was main-

tained into adulthood. Although a number of hypothetical relationships between wheel running, corticosterone, and growth can be suggested, our data, which are descriptive in nature, do not allow us to distinguish among them. One possibility is that reduction in growth rate and elevated plasma corticosterone are each correlated to responses of selection for high wheel running but are not causally related to each other. Arguing against this possibility is the fact that the negative relationship between growth and corticosterone levels was significant within each of the four groups, including the control animals not under selection for wheel running.

Instead, we suggest that wheel running, corticosterone levels during activity, and suppression of growth may be linked at the level of the central nervous system. As proposed above, one potential mediator of such a relationship is CRF, which affects energy balance and release of growth hormone. CRF is thought to play an important role in the control of food intake and energy expenditure independently of its actions on the HPA axis. Centrally administered CRF decreased food intake and body mass in rats (4) and decreased food intake in fat-tailed dunnarts (*Sminthopsis crassicaudata*; Ref. 23) and goldfish (*Carassius auratus*; Ref. 15). Hypersecretion of CRF may also be responsible for weight loss in anorexia nervosa (25). Additionally, stress also inhibits secretion of growth hormone in rats (36), perhaps through the action of CRF neurons. Central administration of CRF in rats decreased growth hormone secretion in a dose-dependent manner (35), and stress-induced decreases in plasma growth hormone concentrations are blocked by pretreatment with the CRF antagonist α-helical $CRF_{9–41}$ (39).

It is also possible that voluntary wheel running (and maybe voluntary home-cage activity) is causally related to both elevated corticosterone and reduced growth through separate pathways. Clearly the HPA axis responds acutely to exercise and causes elevated plasma corticosterone levels. Suppression of growth in exercising animals could result from limited energy availablility or through alterations in hormone pathways other than the HPA axis. Energy deficits from insufficient food or forced exercise can result in reduced body mass (5). In rats, daily voluntary running reduced fat, protein, and body mass in young males (but not females), possibly through the involvement of gonadal hormones (13). Obviously, level of running and selection history are confounded in our study, and we are prevented from any substantive discussion of the relationships of wheel running, corticosterone, and growth. Experimental studies, perhaps with adrenalectomized animals or with manipulation of CRH levels, will be required to elucidate this interesting finding.

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