Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats

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Fediuc, Sergiu, Jonathan E. Campbell, and Michael C. Riddell. Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. J Appl Physiol 100: 1867–1875, 2006. First published January 26, 2006; doi:10.1152/japplphysiol.01416.2005.—Adaptations of the hypothalamic-pituitary-adrenal (HPA) axis to voluntary exercise in rodents are not clear, because most investigations use forced-exercise protocols, which are associated with psychological stress. In the present study, we examined the effects of voluntary wheel running on the circadian corticosterone (Cort) rhythm as well as HPA axis responsiveness to, and recovery from, restraint stress. Male Sprague-Dawley rats were divided into exercise (E) and sedentary (S) groups, with E rats having 24-h access to running wheels for 5 wk. Circadian plasma Cort levels were measured at the end of each week, except for week 5 when rats were exposed to 20 min of restraint stress, followed by 95 min of recovery. Measurements of glucocorticoid receptor content in the hippocampus and anterior pituitary were performed using Western blotting at the termination of the restraint protocol. In week 1, circadian Cort levels were twofold higher in E compared with S animals, but the levels progressively decreased in the E group throughout the training protocol to reach similar values observed in S by week 4. During restraint stress and recovery, Cort values were similar between E and S, as was glucocorticoid receptor content in the hippocampus and pituitary gland after death. Compared with E, S animals had higher plasma ACTH levels during restraint. Taken together, these data indicate that 5 wk of wheel running are associated with normal circadian Cort activity and normal negative-feedback inhibition of the HPA axis, as well as with increased adrenal sensitivity to ACTH after restraint stress.

voluntary exercise; circadian rhythm; glucocorticoids; adrenocorticotrophic hormone; glucocorticoid receptor; hypothalamic-pituitary-adrenal axis

DYSREGULATION AND HYPERRESPONSIVENESS of the hypothalamic-pituitary-adrenal (HPA) axis have been linked to a number of pathological conditions such as increased insulin resistance (45), increased adiposity (34), immune suppression (28, 36), depression (18), osteoporosis (37), muscle wasting (21), and cardiovascular disease (29, 44). Patients with Cushing’s syndrome, a disease caused by oversecretion of glucocorticoids (GC), and individuals exposed to chronic stress have some, if not all, of these conditions, suggesting that elevations in circulating GC have widespread health implications (7, 35, 38).

Alternatively, regular exercise (i.e., training) has been associated with many positive effects in body function such as improved insulin sensitivity (33), decreased visceral adipose tissue mass (17), and improvements in cardiovascular function (24). Interestingly, exercise is considered a common form of physiological stress that is associated with increased levels of GC released into the circulation (39).

Although adaptations of the HPA axis to exercise have been studied extensively, most investigations using rodents employ forced-exercise training protocols, such as swimming and treadmill running (6, 22, 25, 30, 42). One key characteristic of forced exercise is that the animals experience a loss of control over their activity pattern, often made to run or swim during the lights-on cycle, thereby causing severe psychological stress and major disruption in the normal circadian rhythm of the HPA axis. Although this kind of exercise can lead to positive adaptations like increased oxidative enzyme activity and decreased body mass, it is also associated with negative physiological adaptations such as increased adrenal weight and thymic involution (30). Moreover, rodents in forced treadmill studies are often stimulated to run with electrical foot shocks that also cause a significant stress response (30). Voluntary wheel running, on the other hand, allows rodents to exercise at their own volition during the lights-off phase when they are naturally most active.

To date, only a limited number of studies have investigated the effects of voluntary wheel running on circadian GC activity. Droste et al. (11) recently reported the circadian rhythm of the HPA axis after 4 wk of voluntary wheel running in mice. Wheel exposure was characterized by a large increase in plasma corticosterone (Cort) levels at the onset of the dark cycle in exercising mice at the end of the training protocol. However, it is unknown whether circadian adaptations to Cort release occurred throughout the training protocol. This points to a need for additional research to elucidate the circadian GC adaptation to voluntary wheel running in rodents.

An additional area of controversy is the influence of exercise training on the HPA axis response to a novel stressor. In rats, activity wheel running for 6 wk does not alter the Cort and ACTH responses to electrical foot shock compared with sedentary animals (9). In a follow-up study (8), these same investigators used a similar 6-wk wheel running protocol and examined the adrenocortical responses to foot shock and cage switching. They found attenuated ACTH responses but similar Cort levels to a combination of both novel stressors in animals exposed regularly to running wheels, compared with sedentary animals. These observations suggest that long-term wheel running is associated with a lower central HPA activation but increased adrenal sensitivity to ACTH, thus resulting in a normal GC levels in response to a nonexercise stress. Similarly, Droste et al. (11) observed that mice exposed to wheel running experience increased adrenal sensitivity to ACTH in...
response to both restraint stress and forced swimming (11). However, whereas these investigations show the effects of voluntary wheel running on the HPA axis response to a novel stressor, neither show the recovery profile after the removal of the stressor. It is precisely this aspect of the stress response that is of particular importance, because impaired negative feedback inhibition of the HPA axis can result in prolonged tissue overexposure to GC.

The purpose of the present investigation was, therefore, to examine the adaptation of the circadian Cort response to long-term voluntary wheel running. In addition, we explored the effects of wheel running on the HPA axis response and, most importantly, the recovery from restraint stress. Additionally, measurements of glucocorticoid receptor (GR) content in the hippocampus and pituitary gland were used to explain any possible differences observed in negative-feedback inhibition of the axis after restraint stress.

METHODS

Animals

Male, Sprague-Dawley rats (age at arrival, 6–7 wk; Charles River Laboratories, Montreal, PQ, Canada) were singly housed in standard, clear, plastic cages (height, 36.4 cm; width, 26.8 cm; depth, 50 cm) and under standard lighting conditions (12:12-h light-dark cycle, lights on at 0600). The temperature and humidity were controlled between 22 and 23°C and between 50 and 60%, respectively. Food (no. 5012 Lab Chows, Ralston Purina, St. Louis, MO) and water were available ad libitum. The body weight and food consumption of the animals were determined daily. Briefly, 50 g of food pellets were placed in each food holder daily. The following day, the remaining food in the food holder was weighed and subtracted from the total amount (50 g) to obtain the food consumed by each animal over 24 h. The animals were handled daily by a single operator to minimize handling stress on the day of the experiment.

Treatment Protocols

On arrival, animals were randomly assigned to either exercise (E) or sedentary (S) groups. After 3 days of habituation to the housing conditions, the E group was given free access to running wheels (wheel circumference, 106 cm; Harvard Apparatus, Holliston, MA) in their cages for a period of 5 wk. A magnetic counter was mounted to each wheel and provided the revolutions after 24 h of use by the animals. Wheel revolutions were recorded daily and then were multiplied by the circumference of the wheel to obtain the distance run by the animals each day. The bedding for each cage was changed once per week. All animal experiments were approved by the York University Animal Care Committee, Toronto, ON, Canada.

Neuroendocrine Experiments

Circadian Cort sampling experiments. Blood samples for circadian plasma Cort concentrations were collected from S and E animals at 1900, 0100, and 0800 at the end of each week of treatment with the exception of week 5. The lights were kept off for the duration of the nighttime blood samples (1900, 1 h after lights off, and 0100) with the exception of a small battery-operated headlamp. For circadian experiments, ~150 µl of blood were obtained by the tail-nick procedure. This blood sampling procedure has been shown not to elevate plasma Cort within the time needed to obtain the sample (15). All samples were obtained within 2 min to minimize HPA-axis activation. The blood was collected in cold heparin-coated capillary tubes (Sarstedt, Montreal, PQ, Canada). All samples were spun in a separate room in a microcentrifuge at 14,000 rpm for 90 s, and the plasma was pipetted into separate tubes and stored at −20°C until assayed.

Animal Surgeries and Restraint Stress Experiments

After completion of the 5-wk treatment period, all animals underwent carotid artery cannulation surgery for the determination of the restraint stress response. Collection of blood samples via a long cannula, as opposed to tail nick, during the restraint stress and recovery is preferred because it allows for enough blood to be collected with minimal animal handling and pain associated with repeated tail sampling (32). Briefly, microcurethane tubing (model MR 40, Braintree Scientific, Braintree, MA) was inserted retrograde into the left carotid artery and secured in place with 3-0 silk. The catheter was tunneled under the skin using a 16-gauge needle and externalized at the nape to the neck. To prevent clotting inside the cannula, a mixture of 40% saline, 40% glycerol, and 20% heparin (wt/vol of 1,000 U/ml) was used. After cannulation surgery, animals recovered in their own cages for a period of 6–10 days, and E animals were permitted to resume wheel running during this time. Subsequent to all restraint stress experiments, the animals were killed by decapitation. The following tissues were extracted and weighed: left and right adrenal glands, left and right plantaris, left epididymal fat pad, hippocampus, and pituitary gland. All tissues were immediately frozen on dry ice, as described previously (32), and stored at −80°C until further analysis.

All restraint experiments were performed between 0900 and 1130, 6–10 days after cannulation surgery at the end of week 5, as previously mentioned. Blood samples were taken for the whole duration of the experiment via cannula to minimize handling stress to the animal. First, the microcurethane cannula was extended by attaching a 1-ft-long PE-50 tube (PE-50, 0.023-in. ID × 0.038-in. OD, VWR International, Mississauga, ON, Canada) to it to allow blood sampling without handling the animal during restraint stress phase of the experiment, as well as recovery phase. A baseline sample was obtained, denoted time 0, after which the animal was placed inside a clear, plastic, aerated tube (Harvard Apparatus, Holliston, MA) for a period of 20 min. A schematic representation of the blood sample during restraint stress and recovery is shown in Fig. 1. After the 20 min of restraint, the animals were taken out of the plastic tube and allowed to move freely in their home cages. The cages were covered with a piece of cardboard (width, 30 cm; length, 55 cm) with a central hole to allow for exteriorization of the cannula outside the cage. The E animals had full access to the running wheels to minimize the stress of a novel environment.

For restraint experiments, the blood was collected in a 1-ml syringe initially, transferred into a 1.5-ml Eppendorf tube and spun down immediately in a separate room from where the experiments took place, to minimize noise and unnecessary stress to the animals. Approximately 100 µl of blood were obtained by cannula for determining Cort concentrations. For determining plasma ACTH concentrations, ~500–600 µl of blood were placed into an Eppendorf tube with 50 µl of an EDTA-Trasylo1 mixture. The contents of the tube were mixed by shaking, then spun down immediately and stored at −20°C until assayed.

Because of the multiple time points sampled (Fig. 1) and the amount of blood necessary for each radioimmunoassay (RIA), addi-
tional blood samples for ACTH were not possible. For the 115 min restraint/recovery protocol, the total blood volume removed from each animal was ~3.5 ml.

Plasma samples for Cort and ACTH were stored at −20 and −80°C, respectively, before being measured by RIA (MP Biomedicals, Costa Mesa, CA). The inter- and intra-assay coefficients of variance for Cort were 7 and 4%, respectively, with a detection limit of 0.4 ng/ml. For ACTH, the inter- and intra-assay coefficients of variance were 7 and 5% respectively, with a detection limit of 2 pg/ml.

**GR Content Analysis**

Western blotting technique was used to analyze tissue expression of GR in the hippocampus and pituitary. For this, tissues were homogenized in lysis buffer (135 mM NaCl, 1 mM MgCl₂, 2.7 mM KCl, 20 mM Tris-base, 0.5 mM Na₂VO₄, 10 mM NaF, 0.2 mM PMSF, 10 μg/ml leupeptin, 1% Triton X-100, and 10% glycerol) and quantified for protein concentration using the Bradford method (3). All samples were then diluted in a 1:1 ratio with Laemmli sample buffer, heated at 95°C for 4–5 min, and then frozen for subsequent analysis.

Acrylamide gels (10%) were prepared using electrophoresis equipment (Bio-Rad, Hercules, CA). Samples previously quantified for protein concentration were thawed on ice and briefly centrifuged before use. The first well of each gel was loaded with 10 μl of molecular weight marker (Full Range Rainbow, Amersham Biosciences, Piscataway, NJ) followed by alternating samples for E and S groups. A quantity of 75 μg of protein was loaded in each well. The samples were run at 110 volts for ~2 h and transferred onto a PVDF transfer membrane (Hybond-P, Amersham Biosciences, Piscataway, NJ) at 4°C and 20 V, overnight. Membranes were blocked for 2 h in 5% milk [Tween 20 Tris-buffered saline (TTBS)] before application of the primary antibody.

For analysis of GR, primary antibody (H-300, Santa Cruz Biotechnology, Santa Cruz, CA) was applied in a 1:500 ratio in 1% milk (TTBS) at 4°C overnight. After the overnight incubation, membranes were washed 3 × 15 min in TTBS buffer and secondary antibody (peroxidase-labeled anti-rabbit, Amersham Biosciences, Piscataway, NJ) was applied in a 1:5,000 ratio in 1% milk (TTBS) for 1 h at room temperature. Next, membranes were washed in TTBS buffer 5 × 10 min. Enhanced chemiluminescence detection (ECL Western Blotting Analysis System, Amersham Biosciences) and film (Bioflex, InterScience) were used to visualize protein content in the dark room. Scion Image, an image processing and analysis program for a windows-based PC, was used to measure the optical density of all bands.

After the analysis of GR, membranes were stripped and reprobed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a mouse monoclonal primary antibody (Abcam, Cambridge, MA), in a 1:5,000 ratio. This antibody was used as a loading control for all membranes. Secondary antibody for GAPDH (peroxidase-labeled anti-mouse, Amersham Biosciences) was used in 1:5,000 ratio.

**Cytochrome-c Oxidase Activity Assay**

Cytochrome-c oxidase (COX) activity in the deep red quadriceps and plantaris muscles was determined as previously described (5). The enzyme activity was determined as the maximal rate of oxidation of fully reduced cytochrome c that is measured by changes in absorbance at 550 nm in a microplate reader (Bio-Tek Instruments). The activity of COX was measured to determine whether the voluntary wheel running protocol resulted in muscle adaptations indicative of training.

**Statistical Analysis**

Statistical procedures employed Statistica software (version 6.0, StatSoft, Tulsa, OK). The body weight, food consumption, adrenal gland size, circadian Cort, restraint Cort, and ACTH were tested for statistical significance by two-way ANOVA. Specifically, body weight, food consumption, circadian Cort, restraint Cort and ACTH were tested by mixed-measures ANOVAs with days or time (min) as the repeated variable. All ANOVAs were followed by contrasts, with the α-level being adjusted using the Bonferroni procedure, in appropriate cases. To determine whether there was violation of sphericity, a Huynh-Feldt correction test was used for all repeated measures in the ANOVAs. Left epididymal fat pad weight, plantaris weight, and GR content in the hippocampus and pituitary gland were tested for statistical significance using Student’s t-tests. P < 0.05 was used as the level of significance in all cases unless indicated.

**RESULTS**

**Physical Parameters**

Figure 2 illustrates the daily food intake over the 6 wk of the investigation. Food intake in both groups increased with respect to time (effect of time, \( P < 0.001 \)). E ate more than S (effect of group, \( P < 0.01 \)); however, the interaction of the two variables (time × group, \( P < 0.001 \)) revealed an initial depression in food intake in the E group in the first 4 days of treatment (\( P < 0.01 \)), followed by a rapid increase to similar levels on day 5. From day 5 to day 14, food consumption remained similar between the two groups. After day 14, food intake diverged between the two groups, with the E animals consuming more food on all days, except days 19 and 21. Cannulation surgery, on day 34, decreased food intake to similar values in both groups 1 day after catheter implantation (day 35). Leading up to the experimental day, food consumption increased at a faster rate in E compared with S.

Figure 3 illustrates the daily body weight, over the 6 wk of the investigation. Both groups had similar body mass at the start of the investigation and gained weight over the course of the study (main effect of time, \( P < 0.0001 \)). A significant time (days) × group interaction (\( P < 0.001 \)) revealed that S gained...
weight at a faster rate than E with values significantly higher in S after the first day.

Figure 4 shows the running distance performed by the E group over the duration of the experiment, including the 6 days after cannulation surgery. The distance increased continuously until day 17 (8,976 ± 999 m), after which it plateaued and remained relatively stable between 8,139 and 1,0277 m. As expected, the day after cannulation, the distance ran by the E group diminished to 1,158 ± 544 m but rebounded toward presurgery levels in the subsequent days.

Tissue weights are shown in Table 1. There was no difference in adrenal mass between groups and left and right side of the body. E animals had less epididymal fat than their S counterparts both in absolute terms ($P < 0.001$) as well as relative to body weight ($P < 0.001$). The weight of the left epididymal fat pad was used as an overall measure of visceral adiposity. Plantaris mass was similar between left and right sides; thus only the left is shown. Plantaris mass was higher in S than in E, when expressed in absolute ($P < 0.001$) but not in relative terms.

**COX Activity Assay**

COX activity (Fig. 5) was assessed in the plantaris and red quadriceps muscles to determine the effectiveness of voluntary wheel running in increasing skeletal muscle oxidative capacity. The plantaris of E animals showed an ~23% increase in COX activity with respect to the S animals ($P < 0.05$). The red quadriceps muscle, however, showed a more pronounced increase in COX activity with an ~80% increase in E animals compared with S animals ($P < 0.001$).

**Neuroendocrine Experiments**

**Circadian plasma Cort variations.** To assess the effects of voluntary exercise on the circadian activity of GC, blood samples were taken at the end of each week of treatment at three different time points (1900, 0100, and 0800) for Cort analysis (Fig. 6). All weeks revealed a significant effect for time of day, characterized by the highest Cort concentrations in the evening (1900) and the lowest concentrations in the morning (0800). Four two-way ANOVAs (group × time of day) illustrate a significant group effect at week 1 ($P = 0.002$; Fig. 6A), week 2 ($P = 0.007$; Fig. 6B), and week 3 ($P = 0.03$; Fig. 6C). Subsequent contrasts for the first 3 wk revealed higher concentrations in the E group at 1900 and 0800 of week 1, at 0100 and 0800 in week 2, and at 0100 in week 3. There were no significant differences in Cort levels at any time point in week 4. There was also no correlation between Cort levels at any time point and running distance during weeks 1–4 (data not shown).

Figure 7 illustrates the chronic effect of voluntary wheel running on plasma Cort during the 4 wk of training (data were pooled across time of day). A two-way ANOVA revealed a significant group effect ($P < 0.01$) and a week × group interaction ($P < 0.05$), illustrating that diurnal Cort of E animals is initially elevated in week 1 and drops continuously over the course of the study. Whereas the initial difference in plasma Cort was relatively large in week 1 (87% greater in E group) and week 2 (90% greater in E group), this difference became increasingly smaller by week 3 (70% difference) and reached similar values in week 4.

**Restraint Stress Experiments**

To assess the effects of 5 wk of voluntary wheel running on the HPA-axis stress response to a novel stressor, both groups of animals were subjected to 20 min of restraint stress (Fig. 8). A two-way ANOVA revealed a significant time effect ($P < 0.001$), indicating that both groups had a robust Cort response to, as well as recovery from, restraint stress (Fig. 8A). A baseline blood sample (time 0) established that both, S and E animals had similar basal Cort levels immediately before restraint stress. The Cort response in the first 20 min of tube restraint did not differ between the two groups at any time point ($P > 0.05$). During recovery, both groups also displayed a similar recovery pattern, reaching baseline values after 80 min of recovery ($P > 0.05$).

Figure 8B illustrates the ACTH response and recovery to 20 min of restraint stress. A two-way ANOVA revealed a significant time × group interaction ($P = 0.02$), illustrating that E...
Table 1. Organ weights for E and S animals

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<td>Plantaris</td>
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Values are means ± SE for 13 exercise (E) and 16 sedentary (S) animals. NS = not significantly different.

animals had an attenuated ACTH response to stress compared with the S animals.

**GR Content in the Hippocampus and Anterior Pituitary**

GR expression was measured in the hippocampus and pituitary gland at the end of the treatment protocol (Table 2). Western blot analyses in both hippocampus and pituitary revealed no difference between E and S animals in GR in either region.

**DISCUSSION**

In the present study, we show that 5 wk of voluntary wheel running is associated with considerable adaptations in circadian plasma Cort and body composition in male Sprague-Dawley rats. The results are highlighted by an initial increase in circadian Cort levels in the E rats, shown in week 1 (Fig. 6), particularly at the lights-off phase of the light-dark cycle when the majority of the wheel running activity is thought to take place (13). This difference gradually decreases over weeks 2 and 3 in the E group to reach similar levels observed in the S animals by week 4 (Figs. 6 and 7), despite a higher running activity (Fig. 4). After 5 wk of wheel running, a 20-min restraint stress experiment revealed no differences in the plasma Cort response, and recovery from a novel stress between the two groups. Interestingly, the similar Cort levels observed in E and S animals for the restraint stress experiment were accompanied by an attenuated increase in plasma ACTH concentration in the E compared with S animals (Fig. 8). Further quantification of GR in the hippocampus and the pituitary, revealed no differences between the two groups of animals (Table 2). Taken together, these data suggest that voluntary wheel running is associated with an initial hyperactivation of the Cort response, followed by a gradual adaptation to exercise, evident from the restored GC levels at the end of week 4. Furthermore, 5 wk of wheel running result in reduced pituitary secretion of ACTH and yet a normal GC response and recovery to restraint, suggestive of an increase in adrenal sensitivity to ACTH.

**Food Consumption, Body Weight, and Running Distance**

In the juvenile rats used in our study, access to a wheel resulted in high-volume, voluntary running. Initially, the animals were exercising only ~1,000 m/day. This distance gradually increased over the next 17 days and remained relatively steady thereafter at ~9,000 m/day until cannulation surgery (Fig. 4). As expected, a dramatic decrease in running distance took place 2 days after cannulation surgery. However, a rapid recovery in running distance to 6,000 m/day, leading up to the restraint experiments illustrates a good recovery from surgery. COX activity, a commonly used marker for training adaptations in muscle (12), was ~20% higher in the plantaris and 80% higher in the red quadriceps of E animals compared with the S animals, despite the decreased running distance after (Fig. 5). Taken together, 5 wk of voluntary wheel running cause significant training adaptations in skeletal muscle aerobic

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**Fig. 5.** Cytochrome-c oxidase (COX) activity between exercise and sedentary animals (n = 8 in each group) in the plantaris (A) and red quadriceps (B) muscles. Values are means ± SE. t-tests revealed higher COX activity in exercise plantaris (*P < 0.05 vs. sedentary) and red quadriceps (#P < 0.001 vs. sedentary) muscles.

J Appl Physiol • VOL 100 • JUNE 2006 • www.jap.org
capacity, despite an interruption in running behavior caused by surgery.

Previous investigations using voluntary wheel running and rats report running distances of 5,000–6,000 m/day (1, 13, 26), which is slightly lower than what we report here for our rats (Fig. 4). The body weight at which animals are introduced to the running wheels appears to influence running volume (2, 40). Rats introduced to running wheels at a body weight of 300–315 run ~5,000 m/day (26), whereas animals in the present experiment were introduced at a body weight of ~175 g, and their running distance plateaued at ~9,000 m/day. Therefore, a low body weight may have been a determining factor for the high distances run by our animals.

Fig. 6. Circadian rhythm of plasma corticosterone between exercise (n = 12) and sedentary (n = 10) animals at the end of each week of training excluding week 5. Values are means ± SE. A 2-way ANOVA was performed on data from each individual week followed by post hoc analysis with contrasts when appropriate. ANOVA results. A: week 1, group effect (P < 0.01), time effect (P < 0.0001). B: week 2, group effect (P < 0.01), time effect (P < 0.0001). C: week 3, group effect (P < 0.05), time effect (P < 0.0001). D: week 4, group effect not significant, time effect (P < 0.0001). Results of the contrasts are represented by * (P < 0.01) and # (P < 0.05) for exercise vs. sedentary animals.

Fig. 7. Circadian plasma corticosterone between exercise (n = 12) and sedentary (n = 10) animals for data pooled across time of day in each group. Samples were obtained at the end of each individual week of training, excluding week 5. Values are means ± SE. A 3-way ANOVA (time of day × week × group) revealed a significant group effect (P < 0.01) and week × group interaction (P < 0.05). Results of the contrasts are represented by * (P < 0.01) and # (P < 0.05) for exercise vs. sedentary animals.

Fig. 8. Plasma hormone response to 20 min of restraint stress. Two-way ANOVAs were used to analyze the data. A: plasma corticosterone in exercise (n = 6) and sedentary (n = 8) animals. The results indicate a significant effect of time (P < 0.0001) but no significant effect of group or time × group interaction. B: plasma ACTH response in exercise (n = 8) and sedentary (n = 11) animals. The results revealed a significant time × group interaction (P < 0.05). Subsequent contrasts revealed higher plasma ACTH concentrations in the sedentary animals at the end of 20 min of restraint stress. *P < 0.05.
The rapid divergence in body weight between E and S animals is in agreement with previous investigations (1, 26). The difference in weight is related to the suppression in food intake (Fig. 2) observed in the E group in the first 4 days of wheel exposure (1, 26). The combination of decreased caloric consumption and increased energy expenditure due to wheel running results in body weight difference within only 2 days of running. This initial suppression of feeding behavior, however, appears to be specific to rats, because other rodents species such as mice (11, 19) or hamsters (16) do not display the same initial pattern of food suppression. It is important to note that the reduction in body mass with wheel running is predominantly caused by a reduction in fat mass and no change in muscle mass, as was evident from plantaris mass expressed relative to body weight (Table 1). The decrease in fat mass is likely a result of increased adipose tissue lipolysis (14). Because GC are known to be a stimulus of abdominal lipolysis (10) it is likely that the initial elevations in HPA-axis activity in the E animals are, at least in part, responsible for the reduction in visceral adipose tissue associated with training.

Diurnal HPA Axis Activity

In the present study, we show that 5 wk of voluntary wheel running initiates a disturbance in GC activity early in the training protocol that is restored by ~4 wk of training. This novel observation is important because it illustrates an adaptive mechanism to limit GC overexposure in target tissues caused by regular exercise. In week 1, circadian plasma Cort is marked by an 87% increase in the E group (Fig. 7). This difference gradually became smaller at the end of week 3 and similar Cort values were reached at the end of the fourth week of training in both E and S animals. Moreover, we failed to observe any increase in adrenal mass as a result of training (Table 1). In contrast to our observations, Droste et al. (11) reported that 4 wk of voluntary wheel running is associated with adrenal hypertrophy and a twofold increase in circulating GC levels at the onset of the dark cycle, similar to what we report here for rats at week 1 of training (Figs. 6 and 7). It may be, therefore, that mice have even higher levels of plasma GC at the start of training that take longer to normalize or that, unlike rats, mice fail to adapt their GC response to training. One limitation of our investigation is that Cort concentrations were not adjusted for blood volume. Part of the decrease in circadian Cort levels observed in the E animals over the course of the investigation may in fact be attributed to a blood volume expansion in response to wheel running. Furthermore, the lack of diurnal ACTH measurements prevented us from determining whether the decrease in Cort levels in trained animals is a result of decreased central activation of the HPA axis or altered adrenal sensitivity to ACTH.

To date, the effect of chronic voluntary exercise on the acute HPA axis response to exercise stress is somewhat controversial. Investigations using forced exercise in rodents have shown that an extended period (4–6 wk) of treadmill or swim training can lead to less robust acute HPA responses to the same absolute exercise stimulus (4, 41, 46). However, if the same relative exercise intensity is performed, rodents (32) and humans (23, 27) generally maintain a high Cort response to the exercise task. Interestingly, we observe, that initially, Cort levels are the highest at the onset of the dark cycle, when rodents tend to have the highest activity levels, but then the level decreases toward sedentary values despite a dramatic increase in running volume. Additionally, we also observed in E animals reductions in Cort levels both at lights on (0800) and during the nighttime hours (0100). Because running distance increased during the course of the investigation, the lower Cort levels in E animals by week 4 may be indicative of training adaptations or acclimatization to the novelty of running behavior. Although we are not certain of the mechanism for the attenuated circadian GC levels during training, we believe that there may be a gradual reduction in corticotropin-releasing hormone (CRH) release, or a decrease pituitary sensitivity to CRH stimulation, the later of which has been reported with training in humans (27).

Restraint Stress Experiments

In light of the gradual decrease in circadian Cort levels observed in the E animals over the course of the 5-wk training protocol, we also tested the HPA-axis reactivity of our animals to restraint stress to determine whether their adaptations caused a similar reduction in HPA responsiveness to a novel stressor. Interestingly, 20 min of restraint stress initiated a similar Cort response and recovery in E and S animals (Fig. 8). Because GR protein content in the hippocampus and pituitary, was unaltered between the two groups (Table 2), we believe that negative-feedback inhibition of the axis is normal in E animals after 5 wk of wheel running.

Because daily wheel running causes initial increases in GC secretion, it may be that downregulation in central GR occurs initially, which impairs negative-feedback sensitivity during the first 2–3 wk of wheel running. Indeed, high concentrations of exogenous (20, 31) or exercise-induced release (25) of GCs cause downregulation of GR content in the hippocampus and this is associated with hyperactivation of the HPA axis. Our laboratory has previously shown that rats exposed to daily swimming have a transient decrease in GR mRNA in the PVN and anterior pituitary that coincides with an increase in CRH mRNA (32). In the present study, we show that there are no differences in central GR after 5 wk of training, suggesting that negative feedback sensitivity is intact in these animals. Thus it appears that any initial disturbances in central GR, which may result from the exposure to wheel running, are restored as the training continues.

During restraint stress, we show that E animals had a diminished release of ACTH in response to this novel stress (Fig. 8). Moreover, because the adrenal Cort response in the E animals at the 20-min time point was similar to that of S animals, this suggests an increased adrenocortical sensitivity to ACTH secretion. This finding is supported by previous observations of voluntarily exercising animals experiencing sensiti-
zation to ACTH (8, 11). These observation contrast to what is reported for forced exercise. For example, a previous investigation found that treadmill running in female rats increases ACTH response to foot shock with no differences in Cort response (43). These observations are in line with the finding that treadmill-trained female rats have increased ACTH response to immobilization but similar Cort levels as sedentary animals (42). The reasons for the dissimilar response to a novel stressor in forced vs. volitional training are unknown but may be related to differences in central HPA activation, responses in the adrenal gland, or possibly the sex differences of the animals used in these investigations.

In summary, 5 wk of wheel exposure result in high-volume, voluntary running in male rats, which was accompanied by a decrease in epididymal fat mass. The onset of the training program is characterized by transient increases in circadian Cort levels that last between 2 and 3 wk. After 4 wk of training, any disturbances in circadian plasma Cort are fully restored back to normal despite the maintenance of high volume of wheel running. On completion of the 5-wk voluntary wheel running protocol, 20 min of restraint stress revealed similar plasma Cort concentrations and central GR expression between the two groups, suggesting that central negative-feedback regulation is normal in the exercise animals.

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