Corticosterone administration in drinking water decreases high-fat diet intake but not preference in male rats

Gretha J. Boersma,1 Kellie L. Tamashiro,1 Timothy H. Moran,1,2 and Nu-Chu Liang3

1Department of Psychiatry and Behavioral Sciences, School of Medicine, Johns Hopkins University, Baltimore, Maryland; 2Johns Hopkins Global Obesity Prevention Center, Johns Hopkins University, Baltimore, Maryland; and 3Department of Psychology and Neuroscience Program, University of Illinois-Urbana Champaign, Champaign, Illinois

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Boersma GJ, Tamashiro KL, Moran TH, Liang N-C. Corticosterone administration in drinking water decreases high-fat diet intake but not preference in male rats. Am J Physiol Regul Integr Comp Physiol 310: R733–R743, 2016. First published January 27, 2016; doi:10.1152/ajpregu.00371.2015.—One of the mechanisms through which regular exercise contributes to weight maintenance could be by reducing intake and preference for high-fat (HF) diets. Indeed, we previously demonstrated that wheel-running rats robustly reduced HF diet intake and preference. The reduced HF diet preference by wheel running can be so profound that the rats consumed only the chow diet and completely avoided the HF diet. Because previous research indicates that exercise activates the hypothalamic-pituitary-adrenal axis and increases circulating levels of corticosterone, this study tested the hypothesis that elevation of circulating corticosterone is involved in wheel running-induced reduction in HF diet preference in rats. Experiment 1 measured plasma corticosterone levels under sedentary and wheel-running conditions in the two-diet-choice (high-carbohydrate chow vs. HF) feeding regimen. The results revealed that plasma corticosterone is significantly increased and positively correlated with the levels of running in wheel-running rats with two-diet choice. Experiments 2 and 3 determined whether elevated corticosterone without wheel running is sufficient to reduce HF diet intake and preference. Corticosterone was elevated by adding it to the drinking water. Compared with controls, corticosterone-drinking rats had reduced HF diet intake and body weight, but the HF diet preference between groups did not differ. The results of this study support a role for elevated corticosterone on the reduced HF diet intake during wheel running. The elevation of corticosterone alone, however, is not sufficient to produce a robust reduction in HF diet preference.

WHEEL RUNNING IN RODENTS PROMOTES healthy body weight/composition and can prevent diet-induced or genetic obesity (3, 12, 14, 15, 32, 34, 43). Substantial research has demonstrated reduced food intake during the initial stage of wheel running (5, 7, 18, 24, 35). The food source in these studies included only a single diet, a standard high-carbohydrate (chow) or a high-fat (HF) diet. In recent years, a paradigm of wheel running with two-diet choice, chow vs. HF, available simultaneously has been established (23, 36). We have used this paradigm to demonstrate that wheel running reduces intake and preference to a previously preferred HF diet (23). When the exposure to the HF diet and wheel-running opportunity occurs simultaneously, the reduced HF diet preference can be so profound that the rats avoid the HF diet and consume only the chow diet (29). HF diet avoidance associated with wheel running is robust and lasted even when the rats are no longer running (29). The underlying mechanisms, however, remain obscure.

Multiple studies indicate that wheel running activates the hypothalamic-pituitary-adrenal (HPA) axis (4, 8, 10, 18, 41, 45). Activation of the HPA axis involves the release of corticotropin-releasing factor from the paraventricular nucleus of the hypothalamus that induces the release of adrenocorticotropic hormone from the anterior pituitary gland and the subsequent synthesis and release of glucocorticoids (corticosterone in rodents) from the adrenal gland. Thus elevation of corticosterone can be an index of HPA axis activation. To explore the potential underlying mechanisms of running-associated decrease in HF diet intake and preference, experiments in this study were designed to test the hypothesis that elevation of circulating corticosterone is involved in wheel-running-induced reduction in HF diet preference. Experiment 1 tested the hypothesis by comparing corticosterone levels in sedentary and wheel-running rats provided with simultaneous access to chow and HF diets. If increased release of corticosterone is necessary or sufficient to reduce HF diet preference during wheel running, increased corticosterone should reduce HF diet intake and preference in a sedentary condition. Thus experiments 2 and 3 aimed to test whether increasing corticosterone alone is sufficient to decrease HF diet intake and preference. Circulating corticosterone can be increased by either subcutaneous glucocorticoid injection/pellet or adding corticosterone to the drinking water (25, 28). The latter method is noninnvasive and would be more similar to the situation in running-induced increase in circulating corticosterone, i.e., corticosterone levels are increased after an action, running vs. drinking. Thus experiments 2 and 3 determined whether increasing circulating corticosterone via drinking corticosterone-fortified water would reduce preference to a novel and a previously preferred HF diet, respectively.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley (Harlan, Frederick, MD) rats weighing 250–275 grams upon arrival were the subjects of this study. The rats were housed in a climate-controlled vivarium with a 12-h:12-h light/dark cycle. Rats were individually housed with food and water available ad libitum throughout the experiments. All animal procedures were approved by the Institutional Animal Care and Use Committee at University of Illinois-Urbana Champaign and Johns Hopkins University and in accordance with National Research Council’s Guide for the Care and Use of Laboratory Animals.

Address for reprint requests and other correspondence: Nu-Chu Liang, Dept. of Psychology, Univ. of Illinois-Urbana Champaign, 725 Psychology Bldg., 603 E. Daniel St., M/C 716, Champaign, IL 61820 (e-mail: ncliang8@illinois.edu).
Experiment 1: Wheel Running- and Diet Choice-Associated Corticosterone Levels

Rats were housed individually in conventional tub cages and acclimated to the animal housing room that was maintained at 22–23°C and kept on a 12-h:12-h light/dark cycle (12:00 AM:12:00 PM). They had ad libitum access to water and a standard chow diet (Harlan 2018, 3.1 kcal/g; 24% protein, 58% carbohydrate, 18% fat from soybean oil) during acclimation and the recovery period following jugular vein catheterization surgery. Body weight and food and water intakes were monitored daily throughout the experiment. Data in our laboratory indicate that food spillage consists of constant percentages of daily intake among rats. Thus, in all experiments, it was not measured. The experimental design and timeline are shown in Table 1.

Jugular vein catheterization surgery. After acclimation, rats were anesthetized with a mixture of ketamine (100 mg/kg ip) and xylazine (20 mg/kg ip) for jugular vein catheterization. The jugular vein catheter was made of 10-cm Silastic tubing (no. 508-002; Laboratory Tubing, Midland, MI) with a silicone ring around the tubing about 4.2 cm from one end. This 4.2 cm is about the length from the heart to the insertion point of the jugular vein. After the rats were appropriately anesthetized, fur around the neck was removed, and the top of the skull surface was shaved. An incision was made on the skull surface, and four microscrews were screwed into the skull to form a rectangle around the bregma area. An incision at one side of the neck was made, and after we cleared away fat and connective tissues, the jugular vein was identified. Before implanting the saline-filled Silastic catheter, a 25-gauge needle was used to punch two holes a few millimeters from the same end of the silicone ring. This end with two holes was inserted into the vein and reached the heart. After blood could be drawn successfully from the heart, the Silastic catheter was secured in the jugular vein and then guided around the neck toward the back and placed on the skull surface. A blunted 20-gauge needle was bent to form an elbow shape, and one end of this metal elbow was inserted into the Silastic catheter. Before closing the metal elbow with a cap made of sealed PE-90 tubing, we infused ~0.07 ml of 55% heparinized polyvinylpyrrolidone (PVP40; Sigma Aldrich, St. Louis, MO) into the catheter to maintain the patency of the catheter. This exposed elbow is the point where PE tubing can be connected for the maintenance of the catheter and future blood sampling. The elbow was held in the rectangular space formed by the microscrews on the skull surface, and the position was fixed with dental cement. Skin was sutured closed. During recovery, the jugular vein catheters were maintained by regular flushing with heparinized saline. The procedures for jugular vein catheterization and the catheter maintenance are described in greater detail elsewhere (39).

Diet choice, wheel running, and blood sampling. After the rats recovered from the catheterization surgery (~2 wk), they were divided into three groups. The wheel-running group (WRChoice) was individually housed in a cage with a locked running wheel (Mini Mitter; Philips Respironics, Bend OR). Rats were acclimated to the wheel cages for at least 4 days. On the day of blood sampling, the HF diet (5.24 kcal/g, D12492, 20% protein, 20% carbohydrate, 60% fat from soybean oil and lard; Research Diets, New Brunswick, NJ) was introduced, and wheels were unlocked 2 h before the dark onset. In other words, blood sampling occurred on the first day of HF diet exposure and opportunity to run in the wheels. Wheel-running activity was imported to a computer, and postrecording analysis was done with VitalView data acquisition system (Respironics, Murrysville, PA). There were two control groups. The first sedentary (Sed) group served as naïve control, and the rats continued to be housed individually in the conventional tubs with water and the chow diet available ad libitum (SedChow). In addition to the chow diet, the second Sed group received the 60% HF diet 2 h before the dark onset on the day of blood sampling (SedChoice). The inclusion of SedChow and SedChoice without WRChoice as control groups was based on previous reports that wheel running increases corticosterone levels in a chow-fed condition (4, 10) and that a limited amount of HF diet was consumed on the first day of wheel running in a chow vs. HF diet regimen (29). The experiments thus focused on the potential role of corticosterone in the reduction of HF diet intake.

On the sampling day, the first sample (~200 μl) was taken from the jugular vein catheter at 7:00 AM, and subsequently a sample was taken every 2 h. The first blood sample was considered baseline and was not included in data analysis. For the SedChoice and WRChoice rats, the HF diet was introduced, and wheels were unlocked 1 h after the second sample (10:00 AM). Twelve samples were taken in total, and the volume of blood collected was <10% of the body weights of the rats. After blood was collected, it was transferred into a K2EDTA-coated tube (no. 365974; BD Diagnostics, Franklin Lakes, NJ), placed on ice, and centrifuged at 3,000 revolution/min at 4°C for 15 min. Plasma was collected into plastic microcentrifuge tubes and stored at ~80°C for future analysis.

Experiment 2: Simultaneous Exposure of HF Diet and Corticosterone in Drinking Water

Rats were acclimated to the animal housing room that was maintained at 22–23°C and kept on a 12-h:12-h light/dark cycle (8:00 AM:8:00 PM) with ad libitum access to water and a standard chow diet (Harlan 2018, 3.1 kcal/g) for 7 days. Body weight and food and water intakes were recorded daily throughout the experiment. Experimental design and timeline are shown in Table 1.

Blood collection for plasma corticosterone levels. To measure the circadian nadir and peak of circulating corticosterone, blood samples were collected via tail nick into heparinized capillary tubes, transferred to microcentrifuge tubes, placed on ice, and centrifuged at

Table 1. Group design and timeline for experiment 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Habituation, ~1 wk</th>
<th>Surgery and Recovery, ~2 wk</th>
<th>Acclimation, &gt; 4 days</th>
<th>Jugular Vein Blood Sampling, once every 2 h in 1 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SedChow</td>
<td>Housed in tubs with ad lib access to water and chow diet</td>
<td>Jugular vein catheterization surgery and recovery. All rats housed in tubs with ad lib access to water and chow diet</td>
<td>Housed in tubs with ad lib access to water and chow diet</td>
<td>Housed in tubs with ad lib access to water and chow diet</td>
</tr>
<tr>
<td>SedChoice</td>
<td></td>
<td></td>
<td></td>
<td>Wheels remain locked and HF diet introduced 2 h before dark onset</td>
</tr>
<tr>
<td>WRChoice</td>
<td></td>
<td></td>
<td></td>
<td>Wheels unlocked and HF diet introduced 2 h before dark onset</td>
</tr>
</tbody>
</table>

HF, high-fat diet; SedChow, sedentary group on the chow diet; SedChoice; sedentary group with a choice of chow diet or HF diet; WRChoice, wheel-running group with choice of chow diet or HF diet.

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CORTICOSTERONE DRINKING AND HIGH-FAT DIET CHOICE

Table 2. Group design and timeline for experiment 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline, 1 wk</th>
<th>Procedures</th>
<th>Diet Choice, 3 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>Ad lib access to water and chow diet</td>
<td>Ad lib access to water and chow diet</td>
<td></td>
</tr>
<tr>
<td>WChoice</td>
<td>Ad lib access to water and chow diet</td>
<td>Ad lib access to water and chow + HF diets</td>
<td></td>
</tr>
<tr>
<td>VehChoice</td>
<td>Ad lib access to vehicle (2% ethanol) solution and chow + HF diets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORTChoice</td>
<td>Ad lib access to corticosterone (in 2% ethanol) solution and chow + HF diets</td>
<td></td>
<td></td>
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</tbody>
</table>

Tail blood samples were collected every 7th day from the last day of baseline. WChoice, group with access to water and both the chow and 60% HF diet; VehChoice; vehicle-choice group with access to 2% ethanol and both the chow and 60% HF diet during the 3-wk treatment period; CORTChoice; group for which water was replaced with a corticosterone solution (400 μg/ml in 2% ethanol).

3,000 revolution/min at 4°C for 15 min. Plasma was collected and stored at −80°C for later analysis of corticosterone using a commercial radioimmunoassay kit (MP Biomedicals, Solon, OH). Blood sampling occurred 30 min after light onset and 1 h before dark onset before the beginning of the experiment (baseline) and every seventh day during the consecutive 3 wk of corticosterone in drinking water treatment. Blood sampling from a tail nick occurred 30 min after the light onset and 1 h before the dark onset. Plasma samples were collected, stored, and analyzed with identical procedures to experiment 2.

Blood collection for plasma corticosterone levels. Similar to experiment 2, blood samples were collected at various time points during this experiment to measure the circadian nadir and peak circulating levels of corticosterone. These time points include baseline, 1 and 10 days after the rats had access to the HF diet, and every seventh day during the consecutive 3 wk of corticosterone in drinking water treatment. Blood sampling from a tail nick occurred 30 min after the light onset and 1 h before the dark onset. Plasma samples were collected, stored, and analyzed with identical procedures to experiment 2.

HF diet choice and corticosterone in drinking water. After baseline blood sampling, a food hopper containing 60% HF diet was introduced to each rat, resulting in simultaneous ad libitum access to a chow and a HF diet. The positions of chow and HF diet hoppers were alternated daily. Once HF diet intake and preference stabilized (15 days), rats were divided into two groups. For the VehChoice (n = 8), water was replaced by 2% ethanol. For the CORTChoice group (n = 9), water was replaced by 400 μg/ml corticosterone in 2% ethanol solution. The ethanol or corticosterone in the drinking water with two-diet choice schedule continued for 3 wk and 2 days (23 days in total). During this period, the drinking solutions were renewed every day. On the last day of the experiment, food was removed 3 h before rats were killed by rapid decapitation at noon (the middle of the light cycle). Trunk blood was collected into K2EDTA-coated tubes (no 366643, BD Diagnostics), placed on ice, and spun at 3,000 revolution/min for 15 min in a refrigerated microcentrifuge. Plasma was collected and stored at −80°C until further analysis of gut peptides, including insulin, peptide YY (PYY), amylin, and glucagon-like peptide-1 (GLP-1). Plasma levels of these peptides were measured to determine whether drinking corticosterone solution affected the se-

Table 3. Group design and timeline for experiment 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Procedures</th>
<th>Diet Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>Ad lib access to water and chow diet</td>
<td>Ad lib access to vehicle (2% ethanol) solution and chow + HF diets</td>
</tr>
<tr>
<td>CORT</td>
<td>Ad lib access to corticosterone (in 2% ethanol) solution and chow + HF diets</td>
<td></td>
</tr>
</tbody>
</table>

Tail blood samples were collected on the last day of baseline and on days 1, 10, 21, 28, and 35.
cretion of gut peptides that are involved in glucose metabolism and the control of food intake (30).

**Plasma Hormone and Peptide Measure**

Plasma corticosterone concentrations were determined by commercially available radioimmunoassay (RIA) kits (MP Biomedicals). Inter- and intra-assay variability for the assay was 6.5–7.1% and 4.4–10.3%, respectively. Plasma samples were divided into several batches for RIA. Each batch of RIA included samples from every group within each individual experiment. Because of an error, samples from two and three rats of the respective VehChoice and COR-TChoice group in experiment 3 were contaminated and thus were excluded from the results. A rat gut hormone multiplex assay (EMD Millipore, Billerica, MA) was used to measure plasma levels of insulin, PYY, amylin, and GLP-1 from trunk blood of rats in experiment 3 with the Luminex machine at the core facility of Johns Hopkins Diabetes Research Center. Inter- and intra-assay precision of the multiplex assay was <24% and <7%, respectively.

**Data Analysis**

Data were analyzed by one-way ANOVA, repeated-measures ANOVA, and post hoc Fisher LSD tests as appropriate using Statistica 7.1 (Tulsa, OK). Chow, HF diet, and total energy intakes were compared in caloric value (kcal). Energy from ethanol (7 kcal/g) was included as part of total energy intake in VehChoice and COR-TChoice rats in experiments 2 and 3. HF diet preference ratio was calculated as HF intake (kcal) divided by HF + chow intake (kcal). Data are presented as means ± SE.

**RESULTS**

**Experiment 1: Wheel Running- and Diet Choice-Associated Corticosterone Levels**

Plasma corticosterone levels in wheel-running rats were significantly higher than levels in the two sedentary control groups. Jugular vein catheters were successfully maintained, and blood samples were collected from six SedChow, seven SedChoice, and nine WRChoice rats. Repeated-measures ANOVA included corticosterone data from 11 sampling time points (Fig. 1A). All groups showed diurnal rhythms of corticosterone levels [the effect of sample: F(10,180) = 9.09, P < 0.0001], i.e., high levels of corticosterone early in the dark cycle and lowest levels of corticosterone immediately after light onset. Plasma corticosterone levels in SedChow and SedChoice groups did not differ, and overall they were both significantly lower than those in WRChoice rats [effects of group and group × sample: F(2,18) = 13.94 and F(20, 180) = 3.04, both P < 0.0003; post hoc vs. WRChoice, P < 0.04]. Post hoc analysis indicated that WRChoice rats had significantly higher baseline corticosterone levels. Thus ANOVA was performed again using data normalized to baseline sample. Whereas group effect showed a trend of higher corticosterone in WRChoice rats [F(2,18) = 2.94, P = 0.08], corticosterone levels were high in the dark cycle and low immediately after light onset [the effect of sample: F(10,180) = 10.37, P < 0.0001]. Furthermore, the effect of group × sample was significant [F(20,180) = 2.38, P < 0.002], and post hoc analysis indicated that corticosterone levels immediately after the dark onset (Fig. 1A, D1) in WRChoice rats were significantly higher than those in SedChow (P < 0.03) and SedChoice (P < 0.0001) rats.

Wheel-running activity was recorded hourly for 24 h after the wheels were unlocked. Most wheel-running activity occurred during the early dark cycle in WRChoice rats (Fig. 1B).

![Fig. 1. Results for experiment 1. A: plasma levels of corticosterone (CORT) during diet choice and wheel running. Time at the light cycle is indicated by L and at the dark cycle by D. The black bar indicates the duration of the dark cycle. In addition to chow, high-fat (HF) diet was introduced for the sedentary group (SedChoice) and wheel-running group (WRChoice), and running wheels were unlocked for the WRChoice rats 1 h after the L9 sample and 2 h before the onset of the dark cycle (L10). CORT levels at various time points were significantly higher in the WRChoice than the sedentary group with chow (SedChow) and SedChoice groups. *SedChow vs. WRChoice, P < 0.05; #SedChoice vs. WRChoice, P < 0.05. B: wheel-running activity for the WRChoice rats during each blood sampling point. C: chow and HF in the 3 groups' diet intake during blood sampling. Letters indicate that chow intakes differ among all 3 groups. #HF diet intake in SedChoice vs. WRChoice, P < 0.05.](http://ajpregu.physiology.org/)

One-way repeated-measures ANOVA revealed a significant effect of sampling time point [F(9,72) = 5.06, P < 0.0001]. A simple regression analysis with corticosterone levels and run-
ning activity from each sampling time point of all WRChoice rats was done to determine the relationship between plasma corticosterone levels and wheel-running activity. The results indicate that plasma corticosterone levels were positively correlated with running activity [F(1,87) = 9.51, P < 0.003; r = 0.31]. Finally, chow and HF diet intakes also differed between groups (Fig. 1C). Intakes of chow in SedChow (57.56 ± 3.3 kcal) rats were significantly more than those in SedChoice (15.06 ± 4.7 kcal) and WRChoice (41.26 ± 0.5 kcal) groups [F(2,19) = 23.7, P < 0.0001]. Two-sample t-test reveals that WRChoice (8.15 ± 2.5 kcal) rats consumed significantly less HF diet than did SedChoice (85.64 ± 8.04 kcal) rats [t(14) = 10.24, P < 0.0001]. Total intake during the blood sampling period in SedChoice rats was significantly higher than that in SedChow and WRChoice rats [F(2,19) = 55.96, P < 0.0001].

**Experiment 2: Simultaneous Exposure of HF Diet and Corticosterone in Drinking Water**

Chow diet intake before the experimental procedures began did not differ among groups. Once HF diet was provided, chow diet intake was significantly reduced and did not differ among the three groups with the two-diet choice feeding regimen [effects of group, time, and group × time: F(2,15) = 1.43, P > 0.2, F(22,330) = 185.74, P < 0.0001, and F(44,330) = 1.34, P = 0.08; Fig. 2A]. Rats in the two-diet choice groups were initially hyperphagic on the HF diet [effect of time: F(20,300) = 46.77, P < 0.0001], and replacing water with ethanol or corticosterone solution did not affect this hyperphagic response [effect of group: F(2,15) = 3.16, P = 0.07; Fig. 2A]. CORTCChoice rats consumed significantly less HF diet than did WChoice and VehChoice rats [effect of group × time: F(40,300) = 1.53, P < 0.03] after 2 wk of drinking corticosterone solution. Nevertheless, HF diet intakes gradually declined in all three groups. All three diet-choice groups showed a gradual decrease in HF diet preference ratio over time [effects of time and group × time: F(20,300) = 14.82, P < 0.0001 and F(40,300) = 1.13, P > 0.2]. Thus, despite lower HF diet intake, HF diet preference ratio in CORTCChoice rats did not differ from that in WChoice and VehChoice rats [effects of group: F(2,15) < 1, P > 0.9; Fig. 2B]. Daily energy intake appeared to be affected by the availability of the HF diet as well as corticosterone consumption (Fig. 2C). In the early stage of the experimental procedure, total energy intake in naïve rats was significantly less than the three groups of rats with two-diet choice [effect of group: F(3,21) = 10.02, P < 0.0003]. As the CORTCChoice rats continued to drink the corticosterone solution, their energy intake became the lowest among the four groups [effects of time and group × time: F(22,462) = 47.37 and F(66,462) = 6.45, both P < 0.0001].

Fluid intake was also affected by the availability of the HF diet as well as corticosterone consumption (Fig. 2D). Repeated-measures ANOVA revealed significant effects of group [F(3,21) = 22.96, P < 0.0001], time [F(22,462) = 9.38, P < 0.0001], and group × time [F(66,462) = 2.96, P < 0.0001]. Water intake did not differ at baseline when all rats had ad libitum access to water and only the chow diet. Consuming HF diet as the primary energy source significantly reduced fluid consumption because fluid intakes in WChoice and VehChoice rats were significantly less than intakes in the naïve group (post hoc vs. naïve, both P < 0.01). Having 2% ethanol as the only fluid source did not affect fluid consumption because fluid intakes of the WChoice and VehChoice groups were stable and did not differ. On the other hand, corticosterone in drinking water treatment further reduced fluid consumption, and fluid intakes in CORTCChoice rats were the lowest among all groups (post hoc CORTCChoice vs. naïve, WChoice, or VehChoice all P < 0.002). Furthermore, daily dosages of ethanol and corticosterone in VehChoice and CORTCChoice rats were calculated based on their daily fluid intakes. VehChoice and CORTCChoice rats respectively consumed 1.2 ± 0.05 and 0.9 ± 0.06 g/kg of ethanol every day. The intake of corticosterone in CORTCChoice rats ranged between 17.7 to 28.8 mg/kg, and on average the dose was 23.0 ± 1.6 mg/kg per day.

It appears that the availability of HF diet, ethanol, and corticosterone solutions all affected body weight (Fig. 2E). Repeated-measures ANOVA revealed significant effects of group [F(3,21) = 17.41, P < 0.0001], time [F(22,462) = 461.18, P < 0.0001], and group × time [F(66,462) = 29.37, P < 0.0001]. Body weight of WChoice rats was significantly higher than the body weight of the naïve (post hoc, P < 0.04) and CORTCChoice (post hoc, P < 0.0001) rats. Drinking 2% ethanol appeared to attenuate the effects of HF diet because body weight of VehChoice was less than that of WChoice (post hoc P = 0.06) and similar to that of naïve. Furthermore, drinking corticosterone solution completely suppressed weight gain in the CORTCChoice group. Body weight in this group was significantly lower than the weight in the other two groups with two-diet choice and also lower than the weight of the naïve group (post hoc CORTCChoice vs. naïve, WChoice, or VehChoice all P < 0.0001).

The results of plasma corticosterone levels on each blood sampling day are listed in Table 4. Repeated-measures factorial ANOVA [group (4) × circadian (2) × day (4)] revealed significant effects of circadian [F(1,21) = 113.04, P < 0.0001], circadian × group [F(3,21) = 39.24, P < 0.0001], circadian × day [F(3,63) = 2.97, P < 0.04], and group × circadian × day [F(9,63) = 4.68, P < 0.0001]. Circulating corticosterone levels immediately after light onset were significantly lower than those before dark onset. Drinking the corticosterone solution appeared to reverse the circadian rhythms of corticosterone levels. That is, corticosterone levels were significantly higher in the CORTCChoice group than the rest of the three groups for the blood samples taken immediately after light onset. Conversely, corticosterone levels were significantly lower in the CORTCChoice group than the rest of the three groups for the blood samples taken before dark onset. Post hoc analysis indicated that naïve rats had significantly higher baseline peak corticosterone levels than those in CORTCChoice rats. Thus ANOVA was performed again using data normalized to baseline (PM samples in Table 4). The analysis indicated significant effects of group [F(3,21) = 7.59, P < 0.002] and group × day interaction [F(9,63) = 2.21, P < 0.04]. Post hoc analysis indicated that corticosterone levels were significantly lower in the CORTCChoice group than those in the controls for samples taken before dark onset (P < 0.02).

**Experiment 3: Corticosterone in Drinking Water After HF Diet Exposure**

Throughout this experiment, chow intake between the two groups remained similar [effects of group and group × time:...
Chow diet intakes in both Veh and CORT groups significantly decreased once HF diet was provided [effect of time: F(38,570) = 57.53, P < 0.0001]. Both groups showed hyperphagia on HF diet during the first few days of the two-diet-choice feeding regimen. When both groups were consuming water, HF diet intakes between Veh and CORT groups did not differ [effect of group: F(1,15) = 3.14, P = 0.1; Fig. 3A]. Two days after water was replaced, CORT rats drinking corticosterone significantly reduced HF diet to an amount lower than their baseline intakes and the amount consumed by Veh rats consuming 2% ethanol [effects of time and group × time: F(36,540) = 32.73 and 1.6,
Finally, among the gut peptides measured by multiplex assay, 0.3], or group and group creased overtime in Veh rats as well [effects of group, time, from that in Veh rats because HF diet preference ratio de-

B ) in CORT rats did not differ significantly than that in Veh rats. The resulting decreased HF diet prefer-

tion for 23 days significantly increased insulin levels [Veh vs. CORT: 1,776.63 ± 262.8 vs. 3,477.65 ± 206.3 pg/ml; t(14)=6.0, P < 0.0001].

### DISCUSSION

Wheel running immediately reduces intake of and preference for a HF diet regardless of its familiarity (23, 29). Results of experiment 1 replicated our previous results that wheel running induces reduced intake of a novel HF diet (29) and demonstrated that plasma corticosterone levels were significantly increased and positively correlated with wheel-running activity during the two-diet-choice feeding regimen. The results are consistent with our hypothesis that elevation of circulating corticosterone may be involved in wheel-running-associated reduction in HF diet intake/preference. Experiments 2 and 3 aimed to further test the hypothesis by determining whether increasing corticosterone without wheel running is sufficient to reduce preference for a novel and a preferred familiar HF diet, respectively. Regardless of familiarity with the HF diet, increasing corticosterone by drinking corticosterone solution reduced HF diet intake, but such reduction was not sufficient to decrease HF diet preference. Results of the three experiments suggest a role for corticosterone in wheel-running-associated decreases in HF diet intake but did not support the hypothesis that increased corticosterone alone is sufficient to induce decrease in HF diet preference.

The result that wheel running significantly increases plasma corticosterone is consistent with previous reports in wheel-running rats and mice (2, 8, 10, 45). Nevertheless, there are novel findings in the current study. Plasma corticosterone levels were measured after certain periods of wheel running, and blood samples were taken at less than three different time points of light-dark cycles in previous studies. The procedures used in this study allowed measuring the changes of corticosterone release before and during wheel running within 1 day, i.e., one light-dark cycle. Although increased corticosterone immediately after other forms of exercise, e.g., treadmill running (13) and forced swimming (1), has been reported, this study demonstrates for the first time that corticosterone release is increased immediately with initiation of voluntary wheel running and that plasma corticosterone levels are positively correlated with the amounts of wheel-running activity.

Furthermore, this is the first study that examines the effects of voluntary wheel running on corticosterone levels with a
chow vs. HF diet feeding regimen. The focus of previous studies was not on determining the effects of wheel running on diet preference, and sometimes the specific type of diet available for the animals was not reported. Except for one study that compared intakes of chow and milk diets (9), rodents were normally maintained with ad libitum access to only one diet, either standard high-carbohydrate chow (10, 45) or a HF diet (2). Plasma corticosterone results in the two sedentary controls suggest that consuming HF diet is unlikely to affect the increase of corticosterone release induced by wheel running. In fact, the reversed preference to the chow and HF diets in SedChoice and WRChoice rats suggests that the running-induced increase in corticosterone may be responsible for the reduced HF diet intake and preference in WRChoice rats.

Adding corticosterone in the source of hydration has been used as a noninvasive method to increase circulating levels of corticosterone. Previous studies in rats (25, 33) and mice (17, 22) have demonstrated that drinking corticosterone solution...
on intakes and body weight are consistent with previous reports. Regardless of administration routes, e.g., subcutaneous pellet (6, 38), daily subcutaneous injections (11, 26, 40), and daily drinking (25, 33), corticosterone treatment significantly reduces fluid and energy intakes and suppresses weight gain in rats. Previous studies have demonstrated that chronic daily administration of a dose of corticosterone (40 mg/kg) higher than the dose consumed by rats in the current study has no significant effects on activity levels in open field test. Thus the reduced intake and body weight are not likely results of malaise or decreased general activity induced by corticosterone (11, 26). Furthermore, short- and long-term corticosterone treatment results in hyperinsulinemia despite low body weight (6, 33, 38), and such effect was also observed in experiment 3. On average, our rats drank ~20 mg/kg of corticosterone each day. This is not a high dose compared with the daily injection of 40 mg/kg used in other paradigms to increase circulating corticosterone (11, 19, 26, 44). Despite the different pharmacodynamics of corticosterone clearance from other routes of administration, oral administration of ~20 mg/kg corticosterone in our experiments is sufficient to alter ingestive behaviors and energy balance. Therefore, the failure of CORTChoice rats to show decreased HF diet preference is not likely due to issues with the route or dose of corticosterone administered to increase circulating corticosterone.

Whereas corticosterone drinking did not significantly reduce HF diet preference, it does reduce HF diet intake and overall daily energy intake, which are similar to the effects of wheel running. The similar effects of wheel running and corticosterone drinking on energy intake support the idea that elevation in corticosterone plays a role in wheel-running-associated reduction in HF diet intake and anorexia. The roles of corticosterone as well as the HPA axis in wheel-running-associated reduction in HF diet intake and preference will need to be further elucidated using approaches that block corticosterone signaling. Furthermore, because corticosterone alone is not sufficient to produce the robust reduction in HF diet preference observed during wheel running, mechanisms other than the HPA axis may be involved. Future studies will need to investigate the roles of other brain neural and hormonal systems, e.g., leptin (31) and orexin signaling (37, 43), in wheel-running-associated reduction in HF diet preference.

**Perspectives and Significance**

An imbalance between exercise/physical activity and consumption of palatable, energy-dense (HF) diet is the main cause of obesity. Appropriate exercise has been considered a good method to prevent and improve health conditions related to weight gain. Although it is clear that exercise can produce negative energy balance to suppress weight gain, its effect on diet choice and underlying mechanisms remain unclear. The current study first demonstrated that voluntary running in rodents reduces HF diet intake and preference, and such intake pattern is accompanied by an increase in circulating levels of corticosterone. Subsequent experiments clarified that elevation of circulating corticosterone alone can suppress weight gain but is not sufficient to produce the robust reduction in HF diet preference seen in voluntary wheel-running condition. Furthermore, various kinds of events, e.g., psychological and physical challenge, can increase the release of stress hormones such as.

### Table 5. Plasma corticosterone levels (ng/ml) during experiment 3

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>Circadian Nadir (AM) vs. Peak (PM)</th>
<th>VehChoice</th>
<th>CORTChoice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>AM 72.38 ± 40.2 PM 18.72 ± 4.3</td>
<td>18.72 ± 4.3</td>
<td>280.81 ± 40.5</td>
</tr>
<tr>
<td>HF D1</td>
<td>AM 69.88 ± 24.4 PM 37.41 ± 18.4</td>
<td>280.81 ± 40.5</td>
<td>319.38 ± 60.8</td>
</tr>
<tr>
<td>HF D10</td>
<td>AM 56.13 ± 29.5 PM 19.83 ± 4.3</td>
<td>280.81 ± 40.5</td>
<td>358.43 ± 123.1</td>
</tr>
<tr>
<td>W1</td>
<td>AM 124.74 ± 36.4 PM 263.69 ± 149.1</td>
<td>319.38 ± 60.8</td>
<td>383.77 ± 102.7</td>
</tr>
<tr>
<td>W2</td>
<td>AM 41.70 ± 15.0 PM 306.12 ± 169.2</td>
<td>358.43 ± 123.1</td>
<td>124.74 ± 36.4</td>
</tr>
<tr>
<td>W3</td>
<td>AM 37.88 ± 11.6 PM 93.41 ± 27.8</td>
<td>358.43 ± 123.1</td>
<td>251.48 ± 30.2</td>
</tr>
<tr>
<td></td>
<td>PM 248.85 ± 14.1</td>
<td>358.43 ± 123.1</td>
<td>248.85 ± 14.1</td>
</tr>
</tbody>
</table>

D. day.
corticosterone to mediate multiple downstream effects, such as increases or decreases in appetite. Thus corticosterone is not considered a catalyst or initiator of “stress” in most conditions. Nevertheless, increased circulating corticosterone via drinking can be considered a psychological stressor because the subject does not experience a physical challenge. The similar effect on body weight but different effects on HF diet preference by corticosterone drinking and voluntary running suggests that the effects of stress challenge on body weight and diet choice are differentiable.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: G.J.B., K.L.K.T., T.H.M., and N.-C.L. designed research; G.J.B. and N.-C.L. drafted manuscript; N.-C.L. interpreted results of experiments; N.-C.L. prepared figures; N.-C.L. analyzed data; G.J.B., K.L.K.T., T.H.M., and N.-C.L. edited and revised manuscript; G.J.B. and N.-C.L. performed experiments; G.J.B. and N.-C.L. interpreted results of experiments; G.J.B. and N.-C.L. analyzed data; G.J.B. and N.-C.L. drafted manuscript; G.J.B. and N.-C.L. interpreted results of experiments; G.J.B. and N.-C.L. designed research; G.J.B. and N.-C.L. drafted manuscript; G.J.B. and N.-C.L. analyzed data; G.J.B. and N.-C.L. performed experiments; G.J.B. and N.-C.L. designed research; G.J.B. and N.-C.L. drafted manuscript.

REFERENCES

31. Patterson CM, Bouret SG, Dunn-Meynell AA, Levin BE. Three weeks of postweaning exercise in DIO rats produces prolonged increases in

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