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

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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

Submitted 24 August 2015; accepted in final form 26 January 2016

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; high-fat diet; diet preference; corticosterone; hypothalamic-pituitary-adrenal axis

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 noninvasive 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.
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 tubes 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 2.

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 80°C for future analysis.
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 for 3 wk after the experimental treatment began.

**Corticosterone in drinking water and HF diet choice.** After baseline blood sampling, rats were divided into four groups with different fluid and diet access for the following 3 wk. On the first day of the experimental procedure, food and water were removed 3–4 h before the dark onset. Two hours before the dark onset, water, 2% ethanol, or corticosterone in 2% ethanol and chow alone or chow and a 60% HF diet were provided to the rats according to their group assignment. To determine whether elevation of peripheral corticosterone levels without wheel running would decrease HF diet preference, water was replaced with a corticosterone solution (400 μg/ml in 2% ethanol) for the corticosterone-choice (CORTChoice) group of rats (n = 6). Based on previous studies, drinking this concentration of corticosterone solution significantly increases plasma corticosterone levels (25, 33). Corticosterone (C2505, Sigma Aldrich) was first dissolved in 100% ethanol and then diluted with water to produce a 2% ethanol solution. Furthermore, in addition to the chow diet, 60% HF diet in a separate food hopper was provided to rats in this CORTChoice group. There were three control groups for this experiment. The naïve group (n = 7) had ad libitum access to water and both the chow and 60% HF diet. The vehicle-choice group (VehChoice; vehicle-choice group with access to 2% ethanol and both the chow and 60% HF diet during the 3-wk treatment period) and VehChoice; group for which water was replaced with a corticosterone solution (400 μg/ml in 2% ethanol).

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).
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 < 0.004]. 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). 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 cor-
related 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
cal) were significantly more than those in SedChoice (15.06 ± 4.7
cal) and WRChoice (41.26 ± 4.05 cal) groups
\([F(2,19) = 23.7, P < 0.0001]\). Two-sample \( t \)-test reveals that
WRChoice (8.15 ± 2.5 cal) rats consumed significantly less
HF diet than did SedChoice (85.64 ± 8.04 cal) 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
\( [\text{effects of group, time, and group × time: } F(2,15) = 1.43, P > 0.2, F(22,330) = 185.74, P < 0.0001, \text{ and } F(44,330) = 1.34, P = 0.08; \text{ Fig. 2A}] \). Rats in the two-diet choice groups were
initially hyperphagic on the HF diet \( [\text{effect of time: } F(20,300) = 46.77, P < 0.0001, \text{ and replacing water with ethanol or corti-
costerone solution did not affect this hyperphagic response}\]
\( [\text{effect of group: } F(2,15) = 3.16, P = 0.07; \text{ Fig. 2A}] \). COR-
TChoice rats consumed significantly less HF diet than did
WChoice and VehChoice rats \( [\text{effect of group × time: } F(40,300) = 1.53, P < 0.03] \) after 2 wk of drinking corticosterone
solution. Nevertheless, HF diet intakes gradually de-
clined in all three groups. All three diet-choice groups showed
a gradual decrease in HF diet preference ratio over time
\( [\text{effects of time and group × time: } F(20,300) = 14.82, P < 0.0001 \text{ and } F(40,300) = 1.13, P > 0.2] \). Thus, despite lower
HF diet intake, HF diet preference ratio in CORTChoice rats
did not differ from that in WChoice and VehChoice rats
\( [\text{effects of group: } F(2,15) < 1, P > 0.9; \text{ Fig. 2B}] \). 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 \( [\text{effect of group: } F(3,21) = 10.02, P < 0.0003] \). As the CORTChoice rats continued to drink the
corticosterone solution, their energy intake became the lowest
among the four groups \( [\text{effects of time and group × time: } F(22,462) = 47.37 \text{ and } F(66,462) = 6.45, \text{ both } P < 0.0001] \).

Fluid intake was also affected by the availability of the HF
diet as well as corticosterone consumption (Fig. 2D). Repeat-
ed-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 CORTChoice rats were the lowest among all groups
\( [\text{post hoc CORTChoice vs. naïve, WChoice, or VehChoice all } P < 0.002] \). Furthermore, daily dosages of ethanol and corti-
costerone in VehChoice and CORTChoice rats were calculated
based on their daily fluid intakes. VehChoice and CORS-
TChoice rats respectively consumed 1.2 ± 0.05 and 0.9 ± 0.06
g/kg of ethanol every day. The intake of corticosterone in
CORTChoice 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 \( [\text{Fig. 2E}] \).
Repeated-measures ANOVA revealed significant effects of group
\( [F(3,21) = 17.41, P < 0.0001, \text{ time } [F(22,462) = 461.18, P < 0.0001, \text{ and group × time } [F(66,462) = 29.37, P < 0.0001] \). Body weight of WChoice rats was significantly
higher than the body weight of the naïve \( [\text{post hoc, } P < 0.04] \)
and CORTChoice \( [\text{post hoc, } P < 0.0001] \) rats. Drinking 2% ethanol appeared to attenuate the effects of HF diet because
body weight of VehChoice was less than that of WChoice \( [\text{post hoc } P = 0.06] \) and similar to that of naïve. Furthermore,
drinking corticosterone solution completely suppressed weight
gain in the CORTChoice 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 \( [\text{post hoc CORTChoice vs. naïve, WChoice, or Veh-
Choice all } P < 0.0001] \).

The results of plasma corticosterone levels on each blood
sampling day are listed in Table 4. Repeated-measures factorial
ANOVA \( [\text{group (4) × circadian (2) × day (4)}] \) revealed
significant effects of circadian \( [F(1,121) = 113.04, P < 0.0001, \text{ circadian × group } [F(3,321) = 39.24, P < 0.0001],
circadian × day \[F(3,63) = 2.97, P < 0.04] \), and group × circadian \[F(9,63) = 4.68, P < 0.0001] \). Circulating
corticosterone levels immediately after light onset were signif-
icantly 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 CORTChoice 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 CORTChoice 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 base-
line peak corticosterone levels than those in CORTChoice rats.
Thus ANOVA was performed again using data normalized to
baseline \( [\text{PM samples in Table 4}] \). The analysis indicated
significant effects of group \( [F(3,21) = 7.59, P < 0.002] \) and
\( \text{group × day interaction } [F(9,63) = 2.21, P < 0.04] \). Post hoc
analysis indicated that corticosterone levels were significantly
lower in the CORTChoice 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 \( [\text{effects of group and group × time:}]} \)
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 $\times$ time: $F(36,540) = 32.73$ and 1.6, $P < 0.05$].

Fig. 2. Results for experiment 2. A: chow and HF diet intakes for the 3 groups with 2-diet choice. At baseline (before day 0), all rats had ad libitum access to only the chow diet. HF diet for the water-choice group (WChoice), vehicle-choice group (VehChoice), and CORT-choice (CORTChoice) groups ($n = 6$/group) was provided on day 1, and at the same time water for the VehChoice and CORTChoice group was replaced by 2% ethanol and 400 $\mu$g/ml CORT in 2% ethanol, respectively. The 2-diet choice and availability of different fluid sources continued for 3 wk. The CORTChoice group had significantly less HF diet intake toward the end of the 3-wk period, and their chow diet intakes were also less than the WChoice group. B: HF diet preference ratio for the 3 groups with 2-diet choice during the 3-wk period did not differ. C: total energy intake for all 4 groups. The naïve group ($n = 7$) had ad libitum access to water and the chow diet throughout the experiment. All 3 groups with HF diet access had significantly more total energy intake during the first few days of 2-diet choice. Overall, rats drinking CORT had the lowest energy intake. D: daily fluid intake was highest in the naïve rats and lowest in the CORTChoice rats. *VehChoice vs. CORTChoice; $WChoice$ vs. CORTChoice; #WChoice vs. VehChoice, $P < 0.05$. E: consumption of CORT in fluid source suppressed weight gain. Body weight of naïve and VehChoice rats was significantly higher than that in CORTChoice rats and lower than that in WChoice rats. *Naïve vs. VehChoice, $P < 0.05$. 

$F(1,15) < 1$ and $F(38,570) = 1.07$, both $P > 0.3$; Fig. 3A]. 

$F(38,570) = 57.53$, $P < 0.0001$. 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 $\times$ time: $F(36,540) = 32.73$ and 1.6, $P < 0.05$]. 

$F(1,15) < 1$ and $F(38,570) = 1.07$, both $P > 0.3$; Fig. 3A].
both $P < 0.02$]. Overall HF diet intake in CORT rats was lower than that in Veh rats. The resulting decreased HF diet preference ratio (Fig. 3B) in CORT rats did not differ significantly from that in Veh rats because HF diet preference ratio decreased overtime in Veh rats as well [effects of group, time, and group $\times$ time: F(1,15) < 1, $P > 0.4$, F(36,540) = 11.6, $P < 0.0001$, and F(36,540) = 1.1, $P > 0.3$]. Similarly, total energy intake (Fig. 3C) did not differ when both groups consumed water, and CORT rats had significantly less daily energy intake after water was replaced by corticosterone solution [effects of group, time, and group $\times$ time: F(1,15) = 7.38, F(38,570) = 25.16 and 2.82, all $P < 0.02$].

Initially water intake was similar between the two groups (Fig. 3D). Fluid intakes were significantly reduced immediately after water was replaced by 2% ethanol and corticosterone solution in Veh and CORT groups, respectively. Fluid intakes increased to the levels of water baseline in Veh rats but remained significantly reduced in the CORT rats throughout the rest of the experimental period [effects of group, time, and group $\times$ time: F(1,15) = 5.88, F(38,570) = 13.29 and 2.59, all $P < 0.01$]. Similarly, differences in body weight occurred soon after water was replaced by 2% ethanol and corticosterone solution, respectively, in Veh and CORT groups (Fig. 3E). Drinking corticosterone solution stopped weight gain in the CORT group and resulted in significantly lower body weight than that of the Veh group [effects of group, time, and group $\times$ time: F(1,15) = 17.98, F(38,570) = 326.02 and 68.37, all $P < 0.001$].

Table 4. Plasma corticosterone levels (ng/ml) during experiment 2

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>Circadian Nadir (AM) vs. Peak (PM)</th>
<th>Naïve WChoice</th>
<th>VehChoice</th>
<th>CORTChoice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>AM 16.08 ± 2.2, PM 15.64 ± 2.2</td>
<td>11.12 ± 4.0,</td>
<td>13.93 ± 5.6,</td>
<td>18.63 ± 6.7,</td>
</tr>
<tr>
<td></td>
<td>W1 236.39 ± 30.8 ab, PM 242.62 ± 30.8</td>
<td>242.62 ± 43.0</td>
<td>219.22 ± 25.6</td>
<td>70.01 ± 29.5</td>
</tr>
<tr>
<td></td>
<td>W2 17.92 ± 2.2, AM 14.22 ± 3.6</td>
<td>25.6a 70.01</td>
<td></td>
<td>215.43 ± 54.5</td>
</tr>
<tr>
<td></td>
<td>W3 236.62 ± 52.4 ab, PM 180.13 ± 42.2</td>
<td>235.39 ± 43.5 a</td>
<td>21.76 ± 6.8 b</td>
<td></td>
</tr>
</tbody>
</table>

Different letters indicate significant differences (post hoc $P < 0.05$) between two groups at the same time point. W, samples taken every 7th day after corticosterone drinking began.

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...
onset or at the middle of light cycle. The results of corticosteroids circadian time points, e.g., at light (nadir) and dark (peak) can significantly increase plasma corticosterone levels at various circadian time points. Plasma corticosterone at circadian nadir in corticosterone-drinking rats in experiment 2 (Table 4) and this result is consistent with previous reports. Plasma corticosterone at circadian peak in corticosterone drinking rats, however, was significantly reduced and less than that in the control rats. On the other hand, corticosterone drinking in experiment 3 resulted in diurnal corticosterone patterns (Table 5) that were different from those in experiment 2 and previous studies (17, 25). Compared with ethanol-drinking rats, plasma corticosterone levels in corticosterone-drinking rats in experiment 3 appeared to become unpredictable, i.e., elevated at some and reduced at the other points. The differences between the two groups were not significant, probably because of large variation in the CORT group. The inconsistent results among this and previous studies are unlikely due to issues with our corticosterone measurement method because normal circadian rhythms of corticosterone levels were demonstrated in naïve, WChoice, and VehChoice groups (Table 4).

Alternatively, plasma corticosterone levels in corticosterone-drinking rats were affected by drinking patterns and the negative feedback mechanism of the HPA axis (19). Eating and drinking occur in close temporal sequence under normal conditions (20, 42). Circadian patterns of feeding can be changed by exposure to HF diet (27) and by the opportunity to choose between different diets (21). The chow vs. HF diet feeding regimen may alter the circadian patterns of eating and drinking. Furthermore, repeated exogenous administration of corticosterone can disrupt normal HPA function and suppress corticosterone release (16). Altered drinking patterns in combination with suppressed endogenous corticosterone release could contribute to lower plasma corticosterone levels detected in corticosterone-drinking rats. Future studies tracking circadian drinking patterns should clarify how circadian patterns of plasma corticosterone in animals drinking corticosterone solution are altered by access to HF diet or HF diet choice.

Although a global elevation of plasma corticosterone levels was not detected in our corticosterone-drinking rats, the effects 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 suggest 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 be 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
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.

ACKNOWLEDGMENTS

The authors thank Laura Moody, Haley York, Brandy Elmore, and Nnamdi Nelson for assistance with these experiments. We also thank Dr. Robert Twining at Marquette University for statistical consulting. Parts of this paper were presented at the 22nd Annual Meeting of the Society for the Study of Ingestive Behavior, Seattle, WA, July 2014.

GRANTS

This research was supported by National Institutes of Health DK-19302 and DK079637 (P30) and the Klarman Family Foundation (to T. Moran) and the startup fund at the Department of Psychology, University of Illinois-Urbana Champaign (to N-C. Liang).

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. conception and design of research; G.J.B. and N-C.L. performed experiments; G.J.B. and 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., K.L.K.T., T.H.M., and N-C.L. approved final version of manuscript; G.J.B. and N-C.L. drafted manuscript.

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