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

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Abstract
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. Since 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. Corticoseterone was elevated by adding it to the drinking water. Compared to 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.

Keywords: wheel running; high fat diet; diet preference; corticosterone; HPA 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). Wheel running associated HF diet avoidance 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 (CRF) from the paraventricular nucleus of the hypothalamus that induces the release of adrenocorticotropic hormone (ACTH) 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 later method is non-invasive
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 or Indianapolis, IN) rats weighing 250-275 g upon arrival were the subjects of this study. The rats were housed in a climate-controlled vivarium with a 12 h on/off, 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 12:12-h light-dark cycle (12:00 AM - 12:00 PM). They had ad libitum access to water and a standard chow diet (Harlan 2018, 3.1kcal/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 is 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 (#508-002, Laboratory Tubing, Midland, MI USA) 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 clearing 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, ~0.07 mL of heparinized 55% Polyvinylpyrrolidone (PVP40, Sigma Aldrich, St Louis, MO USA) was infused 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 weeks), 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, OR, USA). Rats were acclimated to the wheel cages for at least four 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 USA) was introduced and wheels were unlocked 2 hours 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 post recording analysis was done with VitalView data acquisition system (Respiration Inc, Murrysville, PA, USA). 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 two hours before the dark onset on the day of blood sampling (SedChoice). The inclusion of SedChow and SedChoice without WRChow as control groups was based on previous reports that wheel running increases corticosterone levels in a chow fed condition (4, 10) and a limited amount of HF diet was consumed on the first day of wheel running in a chow vs. HF diet feeding 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 hours. 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 hour 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 (# 365974, BD Diagnostics, Franklin Lakes, NJ USA), placed on ice and centrifuged at 3000 RPM at 4° C for 15 min. Plasma was collected into plastic microcentrifuge tubes and stored at -80°C freezer for future analysis.

Experiment 2: Simultaneous exposure of HF diet and CORT in drinking water
Rats were acclimated to the animal housing room that was maintained at 22-23°C and kept on a 12:12-h light-dark cycle (8:00 AM - 8:00 PM) with ad libitum access to water and a standard chow diet (Harlan 2018, 3.1kcal/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 3000 rpm at 4°C for 15 min. Plasma was collected and stored at -80°C freezer for later analysis of corticosterone using a commercial radioimmunoassay kit (MP Biomedicals). Blood sampling occurred 30 min after light onset and 1 hour before dark onset before the beginning of the experiment (baseline) and every 7th day for 3 weeks after the experimental treatment began.

Corticosterone in drinking water and HF diet choice. After baseline blood sampling, rats were divided into 4 groups with different fluid and diet access for the following 3 weeks. On the first day of the experimental procedure, food and water were removed 3-4 hours before the dark onset. Two hours before the dark onset, water, 2% ethanol or corticosterone in 2% ethanol and chow alone or chow + 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 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 3 control groups for this experiment. The naïve group (Naïve, n=7) had ad libitum access to water and two separate food hoppers both containing chow diet. The water-choice group (WChoice, n=6) had ad libitum access to water and both the chow and 60% HF diet. Finally, to distinguish the effects produced by 2% ethanol and corticosterone, the vehicle-choice group (VehChoice, n=6) had ad libitum access to 2% ethanol and both the chow and 60% HF diet during the 3 weeks treatment period. All rats in this experiment had access to a fluid spout and two food hoppers in their
housing cages. The fluids were renewed every day and the positions of the food hoppers were alternated daily to prevent the development of side preference.

**Experiment 3: CORT in drinking water after HF diet exposure**

Two groups of rats were included in this experiment to determine whether elevating corticosterone without wheel running access would decrease preference to a previously preferred HF diet. Rats were acclimated to the animal room that was maintained at 22-23°C and kept on a 12:12-h light-dark cycle (6:00 AM -6:00 PM). They were housed in stainless steel wire mesh hanging cages throughout the experiment. During the acclimation period, all rats had ad libitum access to water and a standard chow diet (Harlan 2018, 3.1kcal/g). Fluid and diet intakes and body weight were recorded daily throughout the experiment. Experimental design and timeline are shown in Table 3.

**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, one and ten days after the rats had access to the HF diet, and every 7th day during the consecutive 3 weeks of corticosterone in drinking water treatment. Blood sampling from a tail nick occurred 30 min after the light onset and 1 hour 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 lib 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 vehicle-choice group (VehChoice, n=8), water was replaced by 2% ethanol. For the CORT-choice group (CORTChoice, 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 weeks 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 hours before rats were sacrificed by rapid decapitation at noon (the middle of the light cycle). Trunk blood was collected into K2EDTA coated tubes (# 366643, BD Diagnostics, Franklin Lakes, NJ USA), placed on ice, spun at 3000 rpm 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 secretion 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 radio immunoassay (RIA) kits (MP Biomedicals, Solon, OH). Inter- and intraassay 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. Due to an error, samples from 2 and 3 rats of the respective VehChoice and CORTChoice group in Experiment 3 were contaminated and thus were excluded from the results. A rat gut hormone multiplex assay (EMD Millipore, Billerica, MA USA) 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 intraassay 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 CORTChoice rats in Experiments 2 & 3. High fat diet preference ratio was calculated as
HF intake (kcal) divided by HF + chow intake (kcal). Data are presented as mean ± standard error of the mean (SEM).

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 6 SedChow, 7 SedChoice, and 9 WRChoice rats. Repeated measures ANOVA included corticosterone data from eleven 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, and 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 (D1 in Fig 1A ) 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 hours after the wheels were unlocked. Most wheel running activity occurred during the early dark cycle in WRChoice rats (Fig 1B). One-way repeated measure 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 running 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 ± 4.05 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 CORT 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 \([effect of time: F(20, 300)=46.77, P < 0.001]\) and replacing water with ethanol or corticosterone solution did not affect this hyperphagic response \([effect of group: F(2, 15)=3.16, P = 0.07; Fig 2A]\). CORTChoice 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 two weeks 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 overtime \([effects of time and group × time: F(20, 300)=14.82, P < 0.0001 and F(40, 300)=1.13, P > 0.2]\). Thus, despite lower HF diet intake, HF diet preference ratio in CORTChoice 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 CORTChoice
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 lib 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 (post hoc CORTChoice vs. Naïve, WChoice or VehChoice all P < 0.002).
Furthermore, daily dosages of ethanol and corticosterone in VehChoice and CORTChoice rats were
calculated based on their daily fluid intakes. VehChoice and CORTChoice 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/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
CORTChoice (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 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 (post hoc CORTChoice 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 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 baseline peak corticosterone levels than those in CORTChoice 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 CORTChoice group than those in the controls for samples taken before dark onset (Ps < 0.02).

Experiment 3: CORT in drinking water after HF diet exposure

Throughout this experiment chow intake between the two groups remained similar [effects of group and group × time: F(1, 15)<1 and F(38, 570)=1.07 both P > 0.3; Fig 3A]. 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, 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 × 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 × 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 × 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 × time: F(1, 15)=17.98, F(38, 570)=326.02 and 68.37, all P < 0.001].

Daily dosages of ethanol and corticosterone were calculated based on the daily fluid intake. On average, daily ethanol consumption was 1.0 ± 0.06 and 0.8 ± 0.05 g/kg, respectively, in Veh and CORT groups. The average corticosterone consumption in CORTChoice rats ranged between 11.7 to 23.4 mg/kg and on average, the dose was 21.1 ± 1.2 mg/kg/day. The results of plasma corticosterone levels on each blood
sampling day are listed in Table 5. Repeated measures factorial ANOVA [group (2) × circadian (2) × day (6)] revealed a significant circadian effect [F(1, 10)=24.31, P < 0.0006] and indicated that circulating corticosterone levels immediately after light onset were significantly lower than those before dark onset. Although it appears that drinking corticosterone solution disturbed the circadian rhythms of corticosterone levels, ANOVA did not reveal significant effects of group × circadian [F(1, 10)=1.4, P > 0.2], group × day [F(5, 50)=1.2, P > 0.3], or group × circadian × day [F(5, 50)=1.21, P > 0.3]. Finally, among the gut peptides measured by multiplex assay, no differences between Veh and CORT groups were found in plasma levels of PYY, amylin, and GLP-1 (data not shown). Two-sample t-test revealed that drinking corticosterone solution for 23 days significantly increased insulin levels [Veh vs. CORT: 1776.63 ± 262.8 vs. 3477.65 ± 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 3 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 one 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 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 non-invasive method to increase circulating levels of corticosterone. Previous studies in rats (25, 33) and mice (17, 22) have demonstrated that drinking corticosterone solution can significantly increase plasma corticosterone levels at various circadian time points e.g. at light (nadir) and dark (peak) onset or at the middle of light cycle. The results
of corticosterone levels in Experiments 2 and 3 were not entirely consistent with those reports. Plasma corticosterone at circadian nadir in corticosterone drinking (CORTChoice) rats was significantly increased and more than that in the three control groups 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 to 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 due to 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 condition (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 CORT 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 is not likely a result 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 to the daily injection of 40 mg/kg used in other paradigms to increase circulating corticosterone (11, 19, 26, 44). In spite of 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 be further elucidated using approaches that block corticosterone signaling. Furthermore, since 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 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. While 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.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

G.J.B., K.L.T., T.H.M., and N-C.L. conception and design of research; G.J.B. and N-C.L. performed experiments; N-C.L. analyzed data; N-C.L. interpreted results of experiments; N-C.L. prepared figures; N-C.L. drafted manuscript; G.J.B., K.L.T., T.H.M., and N-C.L. edited and revised manuscript; G.J.B., K.L.T., T.H.M., and N-C.L. approved final version of manuscript.


Table 1. Group design and timeline for Experiment 1

<table>
<thead>
<tr>
<th>Procedures</th>
<th>habituation (~ 1 week)</th>
<th>surgery &amp; recovery (~2 weeks)</th>
<th>acclimation (&gt; 4 days)</th>
<th>jugular vein blood sampling (once every 2 hours in one day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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 hours before dark onset.</td>
</tr>
<tr>
<td>WRChoice</td>
<td></td>
<td></td>
<td></td>
<td>Wheels unlocked and HF diet introduced 2 hours before dark onset.</td>
</tr>
</tbody>
</table>
Table 2. Group design and timeline for Experiment 2

<table>
<thead>
<tr>
<th>Procedures</th>
<th>baseline (1 week)</th>
<th>diet choice (3 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td>Tail blood samples collected every 7th day from the last day of baseline.</td>
</tr>
<tr>
<td>Naive</td>
<td>Ad lib access to water and chow diet.</td>
<td>Ad lib access to water and chow diet.</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>
</tr>
<tr>
<td>VehChoice</td>
<td>Ad lib access to vehicle (2% ethanol) solution and chow + HF diets.</td>
<td>Ad lib access to corticosterone (in 2% ethanol) solution and chow + HF diets.</td>
</tr>
<tr>
<td>CORTChoice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Group design and timeline for Experiment 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Procedures</th>
<th>baseline (1 week)</th>
<th>diet choice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>water (days 1-14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treatment (days 15-37)</td>
</tr>
<tr>
<td></td>
<td>Tail blood samples collected on the last day of baseline and on day 1, 10, 21, 28, and 35.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>Ad lib access to water and chow diet</td>
<td>Ad lib access to water and chow + HF diets.</td>
<td>Ad lib access to vehicle (2% ethanol) solution and chow + HF diets.</td>
</tr>
<tr>
<td>CORT</td>
<td></td>
<td></td>
<td>Ad lib access to corticosterone (in 2% ethanol) solution and chow + HF diets.</td>
</tr>
</tbody>
</table>
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>Naive</th>
<th>WChoice</th>
<th>VehChoice</th>
<th>CORTChoice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>AM</td>
<td>16.08 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.12 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.93 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.63 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>236.39 ± 30.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>156.75 ± 29.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>175.76 ± 27.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>150.49 ± 41.3&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>W1</td>
<td>AM</td>
<td>17.92 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.92 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.18 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>164.23 ± 32.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>211.92 ± 24.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242.62 ± 43.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>219.22 ± 25.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.01 ± 29.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>W2</td>
<td>AM</td>
<td>16.89 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.22 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.90 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>215.43 ± 54.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>236.62 ± 54.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.13 ± 42.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235.39 ± 43.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.76 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>W3</td>
<td>AM</td>
<td>19.23 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.19 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.91 ± 7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>253.48 ± 55.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>217.02 ± 40.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.49 ± 46.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>213.74 ± 28.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.75 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</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.
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</td>
<td>72.38 ± 40.2</td>
<td>18.72 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>270.31 ± 42.7</td>
<td>280.81 ± 40.5</td>
</tr>
<tr>
<td>HF D1</td>
<td>AM</td>
<td>69.88 ± 24.4</td>
<td>37.41 ± 18.4</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>319.38 ± 60.8</td>
<td>439.77 ± 28.5</td>
</tr>
<tr>
<td>HF D10</td>
<td>AM</td>
<td>56.13 ± 29.5</td>
<td>19.83 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>358.43 ± 123.1</td>
<td>332.50 ± 63.4</td>
</tr>
<tr>
<td>W1</td>
<td>AM</td>
<td>124.74 ± 36.4</td>
<td>263.69 ± 149.1</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>383.77 ± 102.7</td>
<td>149.03 ± 29.5</td>
</tr>
<tr>
<td>W2</td>
<td>AM</td>
<td>41.70 ± 15.0</td>
<td>306.12 ± 169.2</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>251.48 ± 30.2</td>
<td>328.69 ± 289.7</td>
</tr>
<tr>
<td>W3</td>
<td>AM</td>
<td>37.88 ± 11.6</td>
<td>93.41 ± 27.8</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>248.85 ± 14.1</td>
<td>81.88 ± 37.13</td>
</tr>
</tbody>
</table>

D: day; W: samples taken every 7th day after corticosterone drinking began.
FIGURE LEGENDS

Figure 1. Results for Experiment 1. (A) Plasma levels of corticosterone during diet choice and wheel running. Time at the light cycle is indicated by L and at the dark cycle indicated by D. The black bar indicates the duration of the dark cycle. HF diet was introduced for the SedChoice and WRChoice rat and running wheels were unlocked for the WRChoice rats one hour after the L9 sample and two hours before the onset of the dark cycle (L10). CORT levels at various time points were significantly higher in the WRChoice than the 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 three groups diet intake during blood sampling. Letters indicate that chow intakes differ among all three groups. #: HF diet intake in SedChoice vs. WRChoice, P < 0.05

Figure 2. Results for Experiment 2. (A) Chow and HF diet intakes for the three groups with two-diet choice. At baseline (before day 0), all rats had ad lib access to only the chow diet. HF diet for the WChoice, VehChoice and 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 µg/mL CORT in 2% ethanol, respectively. The two-diet choice and availability of different fluid sources continued for 3 weeks. The CORTChoice group had significantly less HF diet intake toward the end of the three weeks period and their chow diet intakes where also less than the WChoice group. (B) HF diet preference ratio for the 3 groups with two-diet choice during the 3 weeks period did not differ. (C) Total energy intake for all four groups. The Naïve group (n=7) had ad lib access to water and the chow diet throughout the experiment. All three groups with HF diet access had significantly more total energy intake during the first few days of two-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. Symbols in (A) to (D), *: 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. *, vs. Naïve and VehChoice, P < 0.05

Figure 3. Results for Experiment 3. The dash line indicates the beginning day that water for the VehChoice and CORTChoice rats was replaced by 2% ethanol and 400 µg/mL CORT in 2% ethanol, respectively. (A) Chow and HF diet intakes for VehChoice and CORTChoice rats. HF diet was introduced on day 1 and such two-diet choice feeding regimen continued for 37 days. HF diet intake in CORTChoice rats significantly reduced after water was replaced by CORT in ethanol. (B) CORT consumption did not change HF diet preference ratio and thus the preference ratio did not differ between groups during the 37 days two-diet choice period. (C) Consumption of CORT significantly reduced daily total energy intake. (D) Fluid intake was reduced after water was replaced by CORT. (E) Consumption of CORT suppressed weight gain and thus body weight in CORTChoice rats was significantly less than that in VehChoice rats. *: Veh vs. CORT, P < 0.05
Fig 1A.

A.

CORT [ng/mL]

Time

L 9 L 11 D 1 D 3 D 5 D 7 D 9 D 11 L 1 L 3 L 5

SedChow, n=6
SedChoice, n=7
WRChoice, n=9
Fig. 1B.

B.

Running Revolution/2 hr

Time
Fig. 1C.

C.

24 hr Intake (kcal)

- Chow
- HF

<table>
<thead>
<tr>
<th>Group</th>
<th>Intake (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SedChow</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>SedChoice</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>WRChoice</td>
<td>40 ± 5</td>
</tr>
</tbody>
</table>

Significance:
- a
- b
- c
- #
Fig. 2B.

B.

Time (day)

HF Diet Preference Ratio

WChoice

VehChoice

CORTChoice
Fig. 2C.

C.

Energy Intakes (kcal/day)

Time (day)

- Naive
- WChoice
- VehChoice
- CORTChoice
Fig. 2D.

D.

- Naive
- WChoice
- VehChoice
- CORTChoice

Fluid Intakes (g/day)

Time (day)

$*$

$*$
Fig. 2E.

**E.**

- Naive
- WChoice
- VehChoice
- CORTChoice

Body Weight (g) vs. Time (day)
Fig. 3A.

A.

Time (day) vs. Intake (kcal/day)

- **Vehchow**
- **VehHF**
- **CORTchow**
- **CORTHF**

**Vehchow** and **VehHF** show a gradual decrease in intake over time, while **CORTchow** and **CORTHF** maintain a higher intake with some fluctuations.
Fig. 3B. Time (day)

HF preference ratio

Veh
CORT
Fig. 3C.

C.

Time (day) 0 4 8 12 16 20 24 28 32 36

Energy Intake (kcal/24hr) 0 20 40 60 80 100 120 140

Veh

CORT

---

** ** **** * **

** * ** **

---
Fig. 3D.

D.

Time (day)

Fluid Intake (ml)

Veh

CORT

---

0 4 8 12 16 20 24 28 32 36
0 10 20 30 40

* * * * * * * * * *
Fig. 3E.
A.

CORT [ng/mL]

- SedChow, n=6
- SedChoice, n=7
- WRChoice, n=9

Time

L 9  L 11  D 1  D 3  D 5  D 7  D 9  D 11  L 1  L 3  L 5
C.

24 hr Intake (kcal)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SedChow</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>SedChoice</td>
<td>c</td>
<td>#</td>
</tr>
</tbody>
</table>

Chow HF
B. Time (day) vs. HF Diet Preference Ratio

- WChoice
- VehChoice
- CORTChoice
E.

- **Naive**
- **WChoice**
- **VehChoice**
- **CORTChoice**

**Time (day)**

**Body Weight (g)**

- 0
- 20
- 40
- 280
- 300
- 320
- 340
- 360
- 380
- 400
- 420

* indicates significant difference.
A.

**Graph A:**
- Intake (kcal/day) vs. Time (day)
- Curves represent different groups:
  - Vehchow
  - VehHF
  - CORTchow
  - CORTHF

Key features:
- Initial sharp decrease in intake
- Steady intake levels thereafter
- Comparison and analysis of intake patterns across groups.