Effect of acute and chronic caloric restriction and metabolic glucoprivation on spontaneous physical activity in obesity-prone and obesity-resistant rats

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Teske JA, Kotz CM. Effect of acute and chronic caloric restriction and metabolic glucoprivation on spontaneous physical activity in obesity-prone and obesity-resistant rats. Am J Physiol Regul Integr Comp Physiol 297: R176–R184, 2009. First published May 6, 2009; doi:10.1152/ajpregu.90866.2008.—Caloric restriction (CR) and metabolic glucoprivation affect spontaneous physical activity (SPA), but it’s unknown whether these treatments similarly affect SPA in selectively bred obesity-prone (OP) and -resistant (OR) rats. OR rats have greater basal SPA and are more responsive to treatments that modulate SPA, such as orexin A administration. We hypothesized that OR rats would be more sensitive to other treatments modulating SPA. To test this, continuous 24-h SPA was measured before and during acute (24 h) and chronic (8 wk) CR in OP, OR, and Sprague-Dawley rats. Pharmacological glucoprivation was produced by injection of 2-deoxyglucose (2-DG), and SPA was measured 5 h postinjection. Acute CR increased SPA in all groups; however, the effect was dependent on the index of SPA and time interval during the 24-h time period. In contrast to OR rats, chronic CR increased distance traveled, ambulatory episodes, and time spent in ambulation and stereotypy during the time interval preceding anticipation of food in OP and Sprague-Dawley rats. Although the effects of 2-DG treatment on SPA were minimal, OR rats had significantly greater SPA than OP and Sprague-Dawley rats independent of treatment. That chronic CR failed to result in significant changes in SPA in OR rats suggests that these rats may be especially unresponsive to treatments modulating feeding. This insensitivity coupled with elevated basal SPA levels may in part mediate phenotypic traits of lean rats.

Food deprivation; locomotor activity; diet-induced obesity

The rising prevalence of obesity indicates that a sustained positive imbalance between energy intake and expenditure exists at the population level. However, it’s unclear which factor, excessive intake or reduced energy expenditure, has contributed more to the obesity epidemic (19). Given the strong influence of genetics and environment on body weight gain, it’s intriguing that spontaneous physical activity (SPA) has a strong familial and genetic component (65) and that lean adults and children display greater SPA than their obese counterparts (25, 38). Similar to lean humans and nonhuman primates (63), rats selected for low weight gain [obesity-resistant rats, (OR)] have greater SPA (57), body-weight adjusted SPA-associated energy expenditure (22), and nonresting energy expenditure (40). The response to treatments affecting food intake also differ between obese and lean rodents: weight gain following consumption of highly palatable diets (30) and weight regain after 50% caloric restriction (CR) is lower in OR rats (35). Further, fat oxidation was elevated in chow-fed OR rats (20). Moreover, total fat pad weight adjusted for body weight was significantly less in OR rats in response to early postweaning exercise (47). Hence, it’s plausible that a divergent response to treatments influencing feeding and physical activity function to perpetuate body weight gain differences in obese and lean rats.

CR reduces total energy expenditure (17, 27), but the effects of CR on individual components of energy expenditure and whether the effect is comparable between obese and lean rodents and humans are less understood (26, 27, 40). In contrast to acute CR, which stimulates physical activity, prolonged CR dampens physical activity levels in rodents (54). There are divergent effects of SPA across species (2, 10–12), and the effect of CR on physical activity is dependent on the duration, severity (14, 54), and age of onset of the CR period (11) in rodents.

We have demonstrated that despite a similar feeding response following intracranial orexin A infusion, OR rats have greater basal and orexin A-induced SPA and elevated brain orexin receptor mRNA compared with obesity prone (OP) rats (57). It is plausible that reduced body weight gain among OR rats may be due, in part, to enhanced responsiveness to agents or manipulations that stimulate physical activity and, in turn, energy expenditure. Therefore, we sought to determine whether OR rats were also more responsive to another stimulii that promotes SPA, CR. We reasoned that like their heightened SPA response to orexin A, OR rats would have an enhanced physical activity response to CR and enhanced protection against obesity. We tested three methods of CR on SPA: 1) acute (24 h) food restriction; 2) chronic food restriction (8 wk); and 3) pharmacological glucoprivation (5 h) induced by 2-deoxyglucose (2-DG), a glucose analog that limits cellular glucose availability.

Another goal of the study was to determine whether feeding-associated activity (FAA), which was first described by Curt Richter [reviewed by (45)] and defined as the increase in locomotor activity prior to a meal (44), was phenotype dependent. FAA, a component of SPA, is reduced in mice lacking orexin neurons, indicating the importance of normal orexin signaling for maintaining FAA (43). Given the potential differences in orexin signaling between OP and OR rats, it is possible that differences in FAA exist between OP and OR rats that may contribute to body weight differences; hence, we determined whether there was a differential effect of CR on FAA between OP and OR rats.

Materials and Methods

Animals

Four-month-old male Sprague-Dawley and selectively bred male OP and OR rats (Charles River, Kingston, NY) were housed individu-
Differentially EFFECT OF CALORIE RESTRICTION ON SPA

usually in cages with a 12:12-h light-dark cycle (lights on at 0700) in a temperature-controlled room (21–22°C). The selectively bred OP and OR rats were obtained commercially from a colony of high-fat-fed outbred Sprague-Dawley rats (Charles River, Kingston, NY) that were selected for their weight gain status. Although a high-fat diet was used during the selective breeding process, consumption of a high-fat diet is not required to observe phenotypic differences in body weight, as OP and OR rats remain obese and lean, respectively, when maintained on a low-fat diet (32, 49). Standard rodent chow (Harlan Teklad 8604) and water were allowed ad libitum except where noted. Studies were approved by the local Institutional Animal Care and Use Committee at the Veterans Affairs Medical Center and the University of Minnesota.

SPA Measurement

SPA was measured using customized, high-precision racks of infrared activity sensors (Med Associates, St. Albans, VT) placed around a square acrylic cage (17.0" × 17.0"), as previously described (57, 59). Briefly, three 16-beam infrared arrays with two arrays in the “x” direction and an elevated “x” array measured ambulation and vertical movement. An activity unit was recorded and time stamped each time a beam was interrupted, and therefore, movement was simultaneously detected in all three axes. Rodent chow was placed on the chamber floor (except where noted), and water was provided in test tubes with stoppers, which were secured in the corners of the chambers two inches from the chamber floor. Thus, vertical movement was not confounded by activity due to eating or drinking. We report several measures of SPA: distance traveled, time spent in ambulatory, (locomotor activity), vertical (rearing or standing on two limbs), and stereotypic movement (small movements, including grooming/feeding) and ambulatory episodes (the number of times movement was initiated).

Specific experimental designs

Study 1. Effect of chronic CR on SPA. Male age-matched rats (n = 10–12/group) were ranked by body weight and then alternatively assigned by rank to a treatment group (ad libitum or chronic CR) to ensure that body weight of the subgroups within a given treatment condition were not significantly different (P = 0.9076). Mean 24-h food intake in the home cage was determined by averaging 24-h food intake for 2 days. Then, 24-h SPA was measured following a 24-h acclimation period in the SPA chambers. Following the 24-h measurement period, rats were returned to their home cages and CR began. Rats in the chronic CR group were given 70% of their mean 24-h food intake plus additional food to account for spillage. Rats in the ad libitum group had ad libitum access to food. Food was given to the chronic CR group daily 2–3 h (1600–1700) before lights off at 1900. The restricted-feeding paradigm was continued until differences in body weight were no longer statistically significant between the calorically restricted OP and ad libitum-fed OR rats, a period of 8 wk (body weight: calorically restricted OP: 505 ± 12.7 and ad libitum-fed OR: 500.7 ± 27.5, P = 0.8813). At that time, 24-h SPA was measured again following a 24-h reacclimation period to the SPA chambers. Body weight was measured weekly, and food intake was measured 2 or 3 times per week. To increase the number of animals in each group and for feasibility, study 1 was repeated in a second group of OP, OR, and Sprague-Dawley rats. Therefore, study 1 was completed in two phases, and the data sets from phases 1 and 2 were combined after it was determined that the 24 h of SPA preceding the restricted-feeding paradigm was not significantly different between rats in phases 1 and 2 for each phenotype (OP: F1,17 = 0.6, P = 0.4307, OR: F1,17 = 2.1, P = 0.1643 and Sprague-Dawley: F1,18 = 1.8, P = 0.1998).

Study 2. Effect of acute CR on SPA. A repeated-measures Latin-square design was used, in which half of the animals in each group (OP, OR, and Sprague-Dawley) received each treatment (ad libitum or acute CR), and both treatments were represented on each treatment day. Therefore, male age-matched rats (n = 10–12/group) were randomly assigned to a treatment group (ad libitum or acute CR) in which body weight of the subgroups within a given treatment condition was not significantly different (P = 0.5986). Rats were acclimated for 24 h to the SPA chambers prior to a continuous 24-h measurement period. Then, rats were returned to their home cages for 72 h with ad libitum access to food and water. Finally, treatments (ad libitum or acute CR) were reversed, and the 24-h acclimation and measurement periods were repeated. Rodents had ad libitum access to rodent chow and water throughout the acclimation and measurement periods, except during the acute 24-h CR measurement period during which food was withheld. Body weight was measured on the first and last treatment day and was used to calculate mean body weight for the study.

Study 3. Effect of 2-DG-induced glucoprivation on SPA. A repeated-measures Latin-square design was used, in which half of the animals in each group (OP, OR, and Sprague-Dawley) received each treatment (vehicle or 2-DG) consistent with study 2. Male age-matched rats (n = 10–12/group) were randomly assigned to a treatment group (saline or 2-DG, 400 mg/kg, dissolved in saline, Sigma, St. Louis, MO), such that body weights of the subgroups within a given treatment condition were not significantly different (P = 0.1026). Rats from study 2 were acclimated to the SPA chambers for 6 h on three separate occasions prior to the start of this study. Rodents had ad libitum access to rodent chow and water throughout the acclimation period. Saline or 2-DG was injected subcutaneously at 1800, 1 h before the start of the dark cycle. Continuous SPA was measured for 5 h postinjection, during which time food was unavailable. Then rats were returned to their home cages for at least 48 h with food and water ad libitum, treatment groups (vehicle or 2-DG) were reversed, and continuous SPA was measured for 5 h postinjection. Body weight was measured on the first and last treatment day and was used to calculate mean body weight for this study.

Statistical Analyses

Data were analyzed using Statview 5.0 (Cary, NC) and are expressed as means ± SE. An alpha level of 0.05 was used for all statistical tests. Data were analyzed by three-factor ANOVA with repeated measures on one (study 1) or two (studies 2 and 3) factors, in which phenotype (OP, OR, Sprague-Dawley) was the between-subjects factor and treatment (study 1: ad libitum or chronic CR; study 2: ad libitum or acute CR; study 3: saline or 2-DG) was the withinsubjects factor. For studies 1 and 2, data were divided into 4-h time bins across the 24-h measurement period and were reported as follows: 0900–1300, 1300–1700, 1800–2100, 2100–0100, 0100–0500, and 0500–0900. In addition, we analyzed data in the cumulative 12-h light cycle, 12-h dark cycle, and the 24-h time interval data. Data from study 3 were analyzed for the 5-h time interval postinjection. A separate analysis was completed for each time interval for the following dependent variables: distance traveled, time ambulatory, time vertical, time stereotypic, and ambulatory episodes). When there was a significant group-by-treatment interaction or when both main effects were significant, a paired-test was completed to determine differences between ad libitum and CR treatments within each group of OP, OR, and Sprague-Dawley rats. Mean body weight (studies 2 and 3), body weight (study 1: initial and body weight change), initial food intake (study 1) were analyzed by one-factor ANOVA followed by Fisher’s paired least significant difference test to determine significant differences between group means.

RESULTS

Study 1. Effect of Chronic CR on SPA

Before the start of the restricted-feeding paradigm, OR rats weighed significantly less than OP and Sprague-Dawley rats, and body weights between OP and Sprague-Dawley rats were

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Mean body weight of OR rats was significantly less than OP (P < 0.0001) and Sprague-Dawley (P < 0.0001) rats, and mean body weight was similar between OP and Sprague-Dawley rats (Table 1). There was a differential effect of acute CR on SPA between groups, which was dependent on the type of SPA and the time interval (Fig. 5). Acute CR increased distance traveled in OR rats (67%) during the 1300–1700 time interval (P = 0.0328) and in OP rats (138%) during 0500–0900 (P = 0.0343). Acute CR increased time vertical in OR (20%) and Sprague-Dawley (29%) rats during the light cycle (P = 0.0238 and P = 0.0086, respectively) and ambulatory episodes in OP rats (18%) during the cumulative 24-h time interval (P = 0.0113). Finally, there was a main effect of treatment on distance traveled, time ambulatory, time vertical, and ambulatory episodes for the cumulative light cycle and the 1300–1700 and 0500–0900 time intervals, which indicates that acute CR increased SPA (P < 0.05, all comparisons, data not shown).

Study 3. Effect of 2-DG-Induced Glucoprivation on SPA

Mean body weight of OR rats was significantly less than OP (P = 0.0001) and Sprague-Dawley (P = 0.0001) rats and mean body weight was similar between OP and Sprague-Dawley rats (Table 1). In general, 2-DG was without effect on SPA with two exceptions (Table 2). In contrast to OP and Sprague-Dawley rats, OR rats had fewer ambulatory episodes in response to 2-DG (vehicle: 177.9 ± 11.3 and 2-DG: 137.9 ± 15.9, P = 0.0446) and 2-DG treatment decreased time stereotypic in all rats (P = 0.0161). Finally, OR rats traveled significantly farther and had greater time ambulatory and time vertical compared with OP and Sprague-Dawley rats independent of treatment (P < 0.05 all comparisons, Table 2).

DISCUSSION

We determined the effect of acute and chronic CR and metabolic glucoprivation on SPA and demonstrate that the effect of CR on SPA not only differed between selectively bred OP, OR, and Sprague-Dawley rats but was also dependent on the method of CR tested, index of SPA measured, and time interval during the 24-h time period. In general, acute and chronic CR stimulated SPA and the effect of 2-DG treatment on SPA was minimal. On the basis of differences in glucosensing between OP and OR rats (28, 34, 36), effects of CR on orexin neurotransmission (21, 39, 53), our previous work showing heightened sensitivity to orexin A following glucoprivation in Sprague-Dawley rats (58, 59) and greater orexin A-induced SPA and orexin receptor mRNA in OR rats (57), we hypothesized that OR rats would be more sensitive to treatments modulating SPA. Instead, chronic CR stimulated SPA in OP and Sprague-Dawley rats and, in general, was without

### Table 1. Body weight and food intake for studies 1 to 3

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<th>Obesity Prone</th>
<th>Obesity Resistant</th>
<th>Sprague-Dawley</th>
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<td>Initial Fl. g</td>
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<td>Mean BW. g</td>
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<td>475 ± 14.6*</td>
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<td><strong>Study Three: Effect of 2-Deoxyglucose on SPA</strong></td>
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<td>Mean BW. g</td>
<td>566 ± 5.9</td>
<td>478 ± 15.5*</td>
<td>566 ± 15.7</td>
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Values are expressed as means ± SE of food intake (FI) and body weight (BW). *P < 0.0001 as compared to obese prone and Sprague-Dawley rats. Initial food intake represents the mean of daily food intake over 2 days prior to starting the restricted-feeding paradigm (n = 9 or 10/group).
effect in OR rats. Effects of acute CR were not confined to a specific group such that the stimulatory effect of acute CR was typically observed in all groups with few group-specific differences noted. Pharmacological glucoprivation reduced stereotypic movement in all groups, but lean OR rats still had significantly greater (baseline) SPA than OP and Sprague-Dawley rats. That OR rats remain lean despite their complete and partial failure to increase SPA in response to chronic and acute CR, respectively, suggests that either this particular group of selectively bred OR rats was at their maximal baseline SPA levels already, they were not necessarily sensitive to all conditions that gave rise to elevated SPA, or as suggested by our previous work, they appear to be specifically sensitive to SPA behavior related to orexin receptor activity. Alternatively, the lack of increase in CR-induced SPA in OR rats may be related to a preferential loss in fat-free mass in response to CR as suggested previously (35), since reduced fat-free mass would be expected to negatively augment SPA. Nonetheless, that lean rats were less responsive to chronic CR than obese rats, despite a comparable rate of weight loss between groups, suggests that insensitivity to this specific feeding-related signal coupled with elevated basal SPA levels in the ad libitum-fed condition together perpetuates the lean phenotype of OR rats. This idea is plausible in the context of reduced food availability, as elevated SPA or foraging in obese rats would increase the probability of finding and consuming food.

Basal SPA, prior to starting the chronic CR-feeding paradigm, was significantly greater in OR rats relative to OP and SD rats, which agrees with and extends our previous findings (57; Fig. 1). Further, the stimulatory effect of CR on SPA shown here is consistent with previous studies demonstrating CR-induced increases in overall physical activity in rodents (2, 11, 13, 18, 64), zebrafish (46), and nonhuman primates (48, 61). Although others have shown no effect of CR on physical activity in rodents (13), nonhuman primates (24), and humans (27), the discrepancies are likely due to methodological inconsistencies between studies since effects of CR on physical activity are species specific and dependent on the duration, severity, modality, and the age of onset of the CR period (2, 11, 24, 41). Unlike mild or intermittent CR, which had no effect on physical activity, severe and continuous CR increased physical activity (54). Additional discrepancies are likely related to the modality used to measure physical activity (18) or the components of physical activity reported (11, 24, 48). Further, the
The final conclusion of studies testing whether CR effects SPA may differ based on the modality and testing environment [e.g., open field chambers vs. running wheels (55) or respiratory chambers vs. free-living condition], time of measurement interval, and the mode of reporting [e.g., total vs. individual components of physical activity or energy expenditure (27, 42, 51)]. For example, CR increased running wheel revolutions during the light cycle but reduced total 24-h wheel revolutions.

**Fig. 3.** Distance traveled (A), time ambulatory (B), time stereotypic (C), and ambulatory episodes (D) during the time interval preceding meal anticipation (1300–1700) in response to chronic caloric restriction, 70% caloric restriction for 8 wk, (shaded bars), or ad libitum feeding (open bars) in OP, OR, and SD rats. *P < 0.05 and #P < 0.005 compared with ad libitum feeding within the same group; n = 9–10/group. Values are presented as means ± SE.

**Fig. 4.** Distance traveled (A), time ambulatory (B), ambulatory episodes (C), time vertical (D), and time stereotypic in response to chronic caloric restriction (E and F), 70% caloric restriction for 8 wk (shaded bars), or ad libitum feeding (open bars) in OP, OR, and SD rats in specific time intervals across the 24-h measurement period. *P < 0.05 compared with ad libitum feeding within the same group; n = 9–10/group. Values are presented as means ± SE.
CREASED SPA DURING THE TIME INTERVAL PRECEDING MEAL ANTICIPATION. OF PARTICULAR INTEREST, WE SHOW THAT CHRONIC CR INDEPENDENT OF THE TIME INTERVAL MEASURED DURING THE 24-H TIME PERIOD. HIGHLY ASSOCIATED WITH CR HIGHLIGHT THE COMPLEXITY OF INTERRESPIRATORY CHAMBERS AND THE FREE-LIVING ENVIRONMENT, BUT WE DID NOT OBSERVE ROBUST EFFECTS OF CR ON 24-H SPA LEVELS DURING THE MEASUREMENT INTERVAL OF THE DARK CYCLE. THIS IDEA IS CONSISTENT WITH THE STIMULATORY EFFECT OF ACUTE AND CHRONIC CR ON FAA BETWEEN OBESE AND LEAN RATS. THE FINDINGS THAT OR, BUT NOT OP, RATS FAILED TO INCREASE FAA IN RESPONSE TO CHRONIC CR AND THAT THE REVERSE WAS TRUE DURING ACUTE CR SUGGEST A DIFFERENTIAL SENSITIVITY TO THIS SPECIFIC FEEDING-RELATED SIGNAL, WHICH COULD PLAUSIBLY PERPETUATE PHENOTYPIC DIFFERENCES IN BODY WEIGHT BETWEEN OP AND OR RATS. THIS IDEA IS CONSISTENT WITH STUDIES SHOWING OP RATS WERE MORE SENSITIVE TO THE ANORECTIC EFFECTS OF CENTRAL INSULIN (8) AND LEPTIN (31, 33) ADMINISTRATION, DESPITE A SIMILAR FEEDING RESPONSE TO OREXIN A (57). ALTERNATIVELY, PERHAPS THE CURRENT GROUP OF OR RATS WERE AT A CEILING LEVEL OF SPA OR THAT THE STRENGTH OF THE STIMULUS (CHRONIC CR) WAS NOT SUFFICIENT TO INCREASE SPA IN THESE OR RATS, SUGGESTING THAT THIS GROUP OF OR RATS COULD NOT BE FURTHER STIMULATED FOR ADDITIONAL SPA BY CHRONIC CR. HOWEVER, WE HAVE OBSERVED A WIDE VARIATION IN SPA BETWEEN GROUPS OF RATS (22). ALTHOUGH OR RATS ARE ALWAYS AT A HIGHER SPA LEVEL THAN OP RATS, THE ABSOLUTE LEVELS VARY CONSIDERABLY, AND WE HAVE PREVIOUSLY OBSERVED GREATER MEAN FAA IN AD LIBITUM-FEED OR RATS, RELATIVE TO THE OR RATS IN THE CURRENT CHRONIC FOOD RESTRICTION CR STUDY. THIS WOULD SUPPORT THE IDEA THAT THE OR RATS IN OUR CURRENT STUDY WERE NOT AT THEIR MAXIMUM SPA LEVELS, AND INSTEAD IT SUGGESTS THAT THE OR RATS SIMPLY WERE LESS SENSITIVE TO THIS FEEDING SIGNAL.

WE PREDICTED THAT 2-DG ADMINISTRATION WOULD FUNCTION LIKE ACUTE CR AND, THEREFORE, STIMULATE PHYSICAL ACTIVITY. WE FURTHER HYPOTHESIZED THAT 2-DG SPA AUGMENTATION WOULD DIFFER BETWEEN GROUPS BASED ON REPORTED DIFFERENCES IN GLUCOSE SENSING BETWEEN OBESE AND LEAN RATS (28, 34, 36), ELEVATED OREXIN RECEPTOR mRNA AND OREXIN A STIMULATION OF SPA IN OR RATS (57), 2-DG ACTIVATION OF OREXIN NEURONS (5) AND AN ELEVATED FEEDING RESPONSE TO OREXIN A FOLLOWING 2-DG ADMINISTRATION (59). IN THIS STUDY, 2-DG, AT A DOSE THAT PREVENTED CELLULAR GLUCOSE UTILIZATION (6) AND INCREASED FOOD INTAKE (9, 16, 50), WAS ADMINISTERED 1 H BEFORE LIGHTS OFF. FOOD WAS UNAVAILABLE DURING THE 5-H TESTING PERIOD POSTINJECTION. DESPITE OUR ATTEMPT TO INDUCE NUTRITIONAL AND PHARMACOLOGICAL GLUCOPRIVATION AND CONTRARY TO OUR EXPECTATIONS, THERE WAS A MINIMAL EFFECT OF 2-DG ON SPA AS 2-DG REDUCED AMBULATORY EPISODES IN OR RATS AND REDUCED TIME IN STEREOTYPY IN ALL RATS. IN AGREEMENT, OTHERS HAVE SHOWN NO EFFECT OF 2-DG-SUPPLEMENTED DIETS OR CHRONIC 2-DG INJECTIONS ON LOCOMOTOR ACTIVITY (41, 60). IT IS PLAUSIBLE THAT WE FAILED TO DETECT AN EFFECT OF 2-DG ON SPA BECAUSE OF THE TIMING OF THE SPA MEASUREMENTS. IN CONTRAST TO THE ACUTE AND CHRONIC CR STUDIES, IN WHICH SPA WAS MEASURED FOR 24 H, SPA WAS MEASURED DURING 5 H OF CR PROMINENTLY DURING THE DARK CYCLE IN THIS STUDY.終於，未観察2-DG刺激SPAは暗時間で測定された基線レベル間の測定時間の暗時間サイクル。このアイデアは、刺激的効果の存在で、急性および慢性CRをONSPAにおいて、光サイクルの少ない日数に、CRの効果なく、異なる影響のない日数に、CRの効果を示す。

THE EVOLUTIONARY ADVANTAGE CONFERRED BY INCREASED SPA FOLLOWING CR IS UNKNOWN, BUT IT IS PLAUSIBLE THAT CR-INDUCED

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**Fig. 5.** Distance traveled (A), time vertical (B), and ambulatory (C) episodes in response to acute caloric restriction (24-h caloric restriction, shaded bars) or ad libitum feeding (open bars) in OP, OR, and SD rats. *P < 0.05 compared with ad libitum feeding within the same group; n = 9–10/group. Values are presented as means ± SE.

(29). Consistent with the aforementioned study, we found CR increased SPA during the light cycle in our open-field environment, but we did not observe robust effects of CR on 24-h SPA. Moreover, previous studies found no effect of CR on SPA measured in respiratory chambers (27, 42), despite a reduction in free-living physical activity level (total energy expenditure adjusted for resting metabolic rate), as determined by doubly labeled water in humans (42). Together, the divergent results noted above and the finding that SPA measured in respiratory chambers and the free-living environment are highly associated (56, 62) highlight the complexity of interpreting studies testing the effect of CR on SPA.

We demonstrate that the SPA-promoting effect of CR was dependent on the time interval measured during the 24-h time period. Of particular interest, we show that chronic CR increased SPA during the time interval preceding meal anticipation [food anticipatory activity (FAA)] in OP and Sprague-Dawley rats but failed to stimulate SPA in OR rats. In contrast, acute CR caused OR rats to travel farther during this time period, while OP and Sprague-Dawley rats were unaffected (Figs. 3A and 5A). Although others demonstrated that CR increased FAA in rodents (11), monkeys (48, 61), and hamsters (1), this is the first report to show a differential effect of acute and chronic CR on FAA between obese and lean rats. The findings that OR, but not OP, rats failed to increase FAA in response to chronic CR and that the reverse was true during acute CR suggest a differential sensitivity to this specific feeding-related signal, which could plausibly perpetuate phenotypic differences in body weight between OP and OR rats. This idea is consistent with studies showing OP rats were more sensitive to the anorectic effects of central insulin (8) and leptin (31, 33) administration, despite a similar feeding response to orexin A (57). Alternatively, perhaps the current group of OR rats were at a ceiling level of SPA or that the strength of the stimulus (chronic CR) was not sufficient to increase SPA in these OR rats, suggesting that this group of OR rats could not be further stimulated for additional SPA by chronic CR. However, we have observed a wide variation in SPA between groups of rats (22). Although OR rats are always at a higher SPA level than OP rats, the absolute levels vary considerably, and we have previously observed greater mean FAA in ad libitum-fed OR rats, relative to the OR rats in the current chronic food restriction CR study. This would support the idea that the OR rats in our current study were not at their maximum SPA levels, and instead it suggests that the OR rats simply were less sensitive to this feeding signal.

We predicted that 2-DG administration would function like acute CR and, therefore, stimulate physical activity. We further hypothesized that 2-DG SPA augmentation would differ between groups based on reported differences in glucose sensing between obese and lean rats (28, 34, 36), elevated orexin receptor mRNA and orexin A stimulation of SPA in OR rats (57), 2-DG activation of orexin neurons (5) and an elevated feeding response to orexin A following 2-DG administration (59). In this study, 2-DG, at a dose that prevented cellular glucose utilization (6) and increased food intake (9, 16, 50), was administered 1 h before lights off. Food was unavailable during the 5-h testing period postinjection. Despite our attempt to induce nutritional and pharmacological glucoprivation and contrary to our expectations, there was a minimal effect of 2-DG on SPA as 2-DG reduced ambulatory episodes in OR rats and reduced time in stereotypy in all rats. In agreement, others have shown no effect of 2-DG-supplemented diets or chronic 2-DG injections on locomotor activity (41, 60). It is plausible that we failed to detect an effect of 2-DG on SPA because of the timing of the SPA measurements. In contrast to the acute and chronic CR studies, in which SPA was measured for 24 h, SPA was measured during 5 h of CR primarily during the dark cycle in this study. Finally, the failure to observe 2-DG-stimulated SPA may be due to a ceiling effect where the dose of 2-DG was insufficient to stimulate SPA above baseline levels during the measurement interval of the dark cycle. This idea is consistent with the stimulatory effect of acute and chronic CR to on SPA during the light cycle, despite no effect of CR during the dark cycle.

The evolutionary advantage conferred by increased SPA following CR is unknown, but it is plausible that CR-induced
SPA represents a purposeful increase in foraging behavior or a heightened motivation to seek food. Therefore, elevated FAA in response to chronic CR in OP and Sprague-Dawley rats may indicate that these rats are more motivated to search for food, which is consistent with operant studies, demonstrating greater food-motivated behavior in obese rats (23). Elevated SPA increases energy expenditure. Therefore, an alternative suggestion is that increased FAA in OP and SD rats occurs in response to interoceptive cues of the obese phenotype to achieve homeostatic energy balance. Irrespective, the differential sensitivity to chronic CR between obese and lean rats has implications for future studies of CR on factors that influence energy balance.

A group of Sprague-Dawley rats was included in each study to determine which group (OP or OR) was more similar to Sprague-Dawley rats. Obesity-prone and -resistant rats were selectively bred from outbred Sprague-Dawley rats. It might be expected that the mean response to CR in Sprague-Dawley rats would lie between that observed in OP and OR rats; however, this was not the case. Body weight and effects of CR on SPA, in general, were most similar between OP and Sprague-Dawley rats, which is consistent with our previous work, showing that body weight and baseline SPA levels in OP and Sprague-Dawley rats were more similar (57). Because Sprague-Dawley rats would be representative of a general population of laboratory rats, these data indicate that the lean phenotype and heightened basal activity levels displayed by OR rats is atypical. As the ability to remain lean is of current difficulty in humans, the neuromolecular underpinnings that govern the lean phenotype of OR rats deserve further investigation.

The mechanism(s) underlying the effects of acute and chronic CR on SPA in OP, OR, and Sprague-Dawley rats is unclear. Caloric restriction increases mRNA and protein levels for orexin (53) and its receptors (21, 39), dopamine receptor signaling (7), and neuropeptide Y, and reduces proopiomelanocortin (4), which augment physical activity. It is possible that the effect of CR on neuropeptide levels differs between these groups of rats, which then contributes to the observed divergent SPA response to CR in OP, OR, and Sprague-Dawley rats. This idea is supported by studies demonstrating differences in brain neuropeptide activity between OP and OR rats. Selectively bred OR rats have elevated orexin one and two receptor mRNA (57), and outbred OP rats have decreased dopamine turnover (37) and dopamine beta hydroxylase, the rate-limiting enzyme required for dopamine synthesis, which modulates physical activity. In addition, hypothalamic projections from the arcuate nucleus of the hypothalamus (ARC), leptin-induced signaling in the ARC, and ARC neurite growth differ between OP and OR rats (3). Hence, these studies suggest CR-induced changes in brain neuropeptide levels likely contribute to the differential effect of CR on SPA between OP and OR rats observed here. Together, our studies indicate there is an unequal effect of acute and chronic CR on SPA between obese and lean rodents and suggest that these effects on SPA may reinforce obesity status.

**Perspectives and Significance**

The widespread prevalence of obesity will continue to challenge society since the environment promotes using labor-saving devices and consumption of energy-dense foods. Theoretically, behavioral therapy targeted toward reducing caloric intake and increasing physical activity-energy expenditure should slow rates of obesity. However, behavioral therapy yields uneven results, which may be due, in part, to the high interindividual variability in weight loss and regain following CR alone or in combination with increased physical activity. Studies of CR in humans indicate that resting energy expenditure is reduced (27). CR in humans also induces a disproportionately large reduction in nonresting energy expenditure, due to increased skeletal muscle work efficiency during low levels of physical activity (52). However, others show that SPA and energy expenditure remained unchanged after 6 mo of CR in humans (42), and nonresting energy expenditure in OR rats was greater than preobese, obesity-prone and weight-reduced rats (40). Although methodological differences between studies likely contribute to divergent results, these studies highlight the need to clarify how CR affects components of nonresting energy expenditure and suggests that differences in metabolic adaptation related to nonresting energy expenditure, specifically low levels of physical activity, may contribute to the high recidivism rate among those formerly obese. Consistent with ideas suggested previously (35) and studies showing effects of CR on changes in fat and fat-free mass (15), the data presented here suggest that compensatory mechanisms in response to CR differ on the basis of body weight phenotype. We found that the magnitude of weight loss in response to CR was similar between lean and obese rats, despite a greater SPA after CR in obese rats, which would be expected to augment nonresting energy expenditure. These data have implications for behavioral therapy and suggest that despite a similar level of CR, the magnitude of SPA required to induce weight loss when combined with CR may be dependent on body weight phenotype.

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REFERENCES


Differential Effect of Caloric Restriction on SPA


