Effect of acute food deprivation on lactational infertility in rats is reduced by leptin administration

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Woodside, Barbara, Alfonso Abizaid, and Shelina Jafferali. Effect of acute food deprivation on lactational infertility in rats is reduced by leptin administration. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1653–R1658, 1998.—The goals of these experiments were to determine whether lactational anestrus would be prolonged by a 48-h fast at days 13 and 14 postpartum (pp) and, if so, to determine whether this effect could be reversed by treatment with the Ob protein leptin. We found that food deprivation on days 13 and 14 pp prolonged lactational infertility by 7 days and that the nutritional experience of both the dam and her litter contributed to this effect. Leptin administration (2.5 mg·kg

r

1 ·day

2

1) during food deprivation was sufficient to reduce the length of lactational infertility compared with vehicle-treated food-deprived rats (P < 0.05). Similar leptin treatment in ad libitum-fed animals reduced food intake (P < 0.05) and litter growth (P < 0.05) but had no statistically significant effect on maternal weight gain or length of lactational infertility. Food-deprived lactating animals had lower circulating leptin levels than ad libitum-fed lactating animals on day 15 pp (P < 0.05), as determined by RIA. Levels in nonlactating rats were higher than in either lactating group (P < 0.05).

FOOD AVAILABILITY PLAYS an important role in the control of reproductive rate in many species (6). If energetic demands are larger than the energy resources available, reproductive function is suppressed until food becomes more abundant. This adaptive mechanism has been illustrated in both field studies (e.g., Refs. 11, 15) and in laboratory experiments in which food restriction or food deprivation is imposed on animals in various reproductive states (see Refs. 26 and 27 for review). For example, food restricting female prepubertal rats delays the onset of their first estrus (6). Similarly, a 48-h food deprivation regimen suppresses luteinizing hormone (LH) levels in adult female rats (7, 16) and disrupts the estrous cycle of female hamsters (24).

During lactation, female mammals show a period of infertility that is also sensitive to energy availability (4, 25). Studies conducted in our laboratory have determined that rat dams that are food restricted to 50% of the ad libitum ration during the first 2 wk of lactation prolong their period of lactational infertility for ~1 wk (29). The extended period of lactational infertility seen after 2 wk of food restriction appears to be related to a prolonged suppression in circulating levels of LH, which has been attributed to a decrease in gonadotropin-releasing hormone (GnRH) release from the hypothalamus (28), and it is not dependent on increases in suckling stimulation from the pups (30). It is not known, however, whether a more acute 48-h fast simi-

lar to that used by Cagampang et al. (7) in rats and by Schneider and Wade (24) in hamsters would also prolong the length of lactational diestrus. Determining the effects of such an acute energy intake manipulation on lactational diestrus would provide more information about the parameters of food restriction necessary to prolong lactational infertility and provide insight into the underlying mechanisms. In experiment 1, therefore, lactating rats were food deprived for 48 h at the end of the second week of lactation and their length of lactational diestrus was compared with that of ad libitum-fed (AL) counterparts. In addition, food-deprived (FD) and AL dams matched for day of parturition had their litters switched to evaluate the contribution of suckling to the length of lactational diestrus seen in the FD groups.

It has been suggested that the GnRH suppression that is observed after periods of food restriction or food deprivation is caused by changes in metabolic fuel availability (24, 26, 27). Animals treated with pharmacological agents that block glucose utilization and fatty acid oxidation, such as 2-deoxy-D-glucose and insulin, show suppressed LH pulses and disrupted estrous cycles (18, 19, 24). More recently, it has become evident that circulating signals from adipose tissue also regulate food intake and reproductive function.

Leptin, the protein product of the ob gene expressed in adipocytes, produces a marked decrease in food intake and body weight in genetically obese and wild-type mice when administered systemically and centrally (8, 12, 20). In addition, leptin treatment accelerates puberty in female mice (9) and participates in the mechanisms that control the onset of puberty in female rats (10). Moreover, systemic leptin treatment corrects some of the reproductive deficits seen in genetically obese mice (ob/ob) (3) and in mice food deprived for 48 h (2). It is therefore reasonable to propose that leptin may act as a signal that could attenuate any effects that food restriction and food deprivation may have on the length of lactational infertility. In experiment 2, we explored the possibility that leptin treatment concurrent with 48 h of food deprivation at the end of the second week of lactation would reverse any effect of fasting on the length of lactational infertility. Finally, because it has recently been reported that leptin levels are low in lactating AL animals relative to nonlactating females (5), we determined the effect of leptin administration on the length of lactational diestrus in AL lactating rats and obtained preliminary data on circulating leptin levels in lactating rats after a 48-h fast as well as in AL lactating and nonlactating rats.

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METHODS AND MATERIALS

Subjects

Virgin female Wistar rats (Charles River Breeding Farms, St.-Constant, PQ, Canada) weighing between 220 and 240 g were used in these experiments. They were housed under controlled conditions (lights on at 0800 and lights off at 2000), at a temperature of 20 ± 2°C, with ad libitum access to food (Agway Lab Chow) and tap water.

General Procedures

Animals were housed in groups of four or five females and one male in each cage. Three weeks later, the females that appeared pregnant were taken out and placed in individual plastic cages (20 × 45 × 50 cm) with Beta Chip bedding. On the day after parturition, day 1 postpartum (pp), litters were culled to eight pups. From day 1 to day 13 pp, the animals had ad libitum access to food and water. Group assignment was carried out on day 13 pp. As of day 4 pp, vaginal smears were taken daily and rated independently by two judges. The smears were rated on the basis of the presence of cornified epithelial cells. The day on which >70% of the cells present on the slide were cornified epithelial cells was assigned as the first estrous day. The length of lactational diestrus was defined as the number of days between parturition and the first estrous day.

Experiment 1. On the morning of day 13 pp, animals were assigned to either the AL or 48-h FD condition, and food was removed from the cages of the rats in the FD group. On the morning of day 15, all animals were fed ad libitum, and the litters of some of the animals in the FD condition were exchanged with age-matched litters of animals in the AL condition, creating four groups: AL dam/AL pups (AL/AL; n = 7), FD dam/FD pups (FD/FD; n = 8), AL dam/FD pups (AL/FD; n = 6), and FD dam/AL pups (FD/AL; n = 6). Litter weight was recorded daily until the end of the study.

Experiment 2. On day 13 pp, animals were assigned to one of three groups: 1) a 48-h FD group (FD + leptin; n = 5) that received intraperitoneal injections of murine leptin (2.5 mg·kg⁻¹·day⁻¹; Preprotech; pH 7.3) every 12 h during the deprivation period, 2) a 48-h FD group (FD + Veh) that received injections of the vehicle (10 mM Tris buffer), and 3) an AL group that also received vehicle injections (AL + Veh). Data collection procedures were as described in experiment 1, with the exception that food intake was also measured during the 2 days that followed the diet-injection regimen (days 15 and 16 pp).

Experiment 3. Animals in this study were assigned either to a leptin-treated (AL + leptin; n = 6) or vehicle-treated (AL + Veh; n = 6) group on day 13 pp. Animals in the AL + leptin group received intraperitoneal injections of murine leptin (2.5 mg·kg⁻¹·day⁻¹; Preprotech; pH 7.35) every 12 h for 48 h beginning at 0800 on day 13 pp. Those in the AL + Veh group received vehicle injections at the same time intervals. Data collection was as described above.

Experiment 4. Nonlactating (n = 7) and lactating (n = 12) females served as subjects in this experiment. The lactating females were assigned to an AL (n = 8) and an FD (n = 4) group. Females in the FD group were food deprived for 48 h beginning on the morning of day 13 pp. Blood samples were taken via indwelling jugular catheters on day 15 pp in the lactating animals and once estrous cyclicity had been established in the nonlactating females, which were fed ad libitum throughout the study. Rats in the FD group were not refed before sampling.

RESULTS

Mean length of lactational diestrus for all four groups is shown in Fig. 1. Overall, FD dams had a longer

Statistical Analyses

Experiment 1. Differences in the length of lactational diestrus among the four groups were assessed using a two-way between-groups ANOVA (with maternal diet condition and pup diet condition as the between-group factors). Differences in dam weight change and litter weight gain measures during and after the deprivation period were evaluated using three-way repeated measures ANOVAs (with maternal diet condition and pup diet condition as the between-group factors and days as the within-group factor).

Experiment 2. A between-groups ANOVA was used to analyze the differences in the length of lactational infertility among the three groups. Food intake measures were averaged to obtain a mean posttreatment (days 16 and 17 pp) score for each group, and these means were compared using a between-groups ANOVA. Repeated-measures ANOVAs (group × day) were conducted to examine the change in dam weight and litter weight during and after the experimental treatment.

Experiment 3. Independent t-tests were used to compare length of lactational diestrus, dam weight change during treatment, and daily pup growth before treatment. Repeated-measures ANOVAs were used to examine the effects of treatment on food intake and pup growth.

Experiment 4. A between-groups ANOVA was used to compare circulating leptin levels among the experimental groups.
period of lactational diestrus than AL dams \[F(1,23) = 28.26, P < 0.05\]. There was no overall effect of nutritional state of the litter \[F(1,23) = 5.0, P = 0.06, not significant\], but there was a significant diet dam \[pups interaction \[F(1,23) = 9.69, P = 0.05\]. Pairwise analyses showed that dams in the AL/AL group had a shorter period of lactational diestrus than any other group and that dams in the FD/FD group had a longer period of lactational diestrus than the dams in the AL/AL and AL/FD groups. The analyses also showed that lactational diestrus was longer in dams in the FD/FD group than in dams in the FD/AL group, although this difference was only marginally significant. Finally, there were no significant differences in the length of lactational diestrus between dams in the FD/AL group and those in the AL/FD group.

As expected, dams in the FD groups (FD/FD and FD/AL) lost weight during the food deprivation period, whereas the AL dams showed a slight increase in body weight during the same period (AL, 9 ± 2.67 g; FD, −94.34 ± 4.1 g). By day 20 pp, FD dams had not gained as much weight as that gained by animals in the AL groups \[F(1,23) = 19.84, P < 0.05\]. Overall, pups in the FD dam groups gained less weight than pups in the AL dam groups \[main effect for maternal diet condition; \[F(8,184) = 87.23, P < 0.05\]. By day 17 pp, all litters were gaining weight at a similar rate \[diet \times time interaction, \[F(8,184) = 76.91, P < 0.05\]. As seen in Fig. 2, both the nutritional history of the dams and that of their litters contributed to the variation in litter growth on days 15 and 16 pp \[diet \times litter switch \times time interaction, \[F(8,184) = 8.30, P < 0.05\]. Simple effects analysis on these days showed that on day 15 pp, pups being nursed by AL mothers gained more weight than pups nursed by FD mothers. In addition, previously FD pups gained more weight than pups nursed by AL dams throughout the experiment. On day 16 pp, litters in the AL/FD group gained more weight than those in any other group.

As expected, dams in the FD groups (FD/FD and FD/AL) lost a significant amount of weight during the deprivation period (AL, 24.95 ± 5.16 g; FD, −97.12 ± 3.67 g) and overall gained less weight than AL dams from day 13 to day 20 pp \[F(2,19) = 28.39, P < 0.05\]. Interestingly, FD dams treated with leptin had not gained as much weight on day 17 pp as...
FD dams treated with vehicle \( [F(2,19) = 10.85, P < 0.05] \) and remained different from AL dams until day 18 pp \( [F(2,19) = 4.22, P < 0.05] \).

Leptin administration had no effect on litter weight change. Litters nursed by FD dams gained less weight during the deprivation period and on days 15 and 16 pp than those nursed by AL dams but gained more weight than litters nursed by AL dams from day 17 to day 19 of lactation [statistically significant group \( \times \) day interaction, \( F(14, 133) = 25.44, P < 0.05 \)].

**Experiment 3**

Mean length of lactational diestrus of the AL + leptin group (20.67 \( \pm \) 0.67 days) was slightly shorter than that of the AL + Veh group (22.67 \( \pm \) 0.99 days) but this difference did not reach statistical significance [t(10) = 1.68, P > 0.05].

Leptin administration had no effect on maternal body weight. Dams in the leptin-treated group lost an average of 3.08 \( \pm \) 4.62 g during treatment, whereas those in the vehicle-treated group lost an average of 1.38 \( \pm \) 2.89 g [t(10) = 0.31, P > 0.05]. As can be seen in Fig. 4A, maternal food intake was reduced by leptin treatment. Although average food intake was similar for both groups before treatment (day 12 pp), females in the AL + leptin group ate less than those in the AL +

**Experiment 4**

Figure 5 shows the average plasma leptin concentrations for AL lactating and FD lactating rats on day 15 pp, together with those of nonlactating rats. Lactating rats had lower levels of circulating leptin than nonlactating rats, and this was further reduced by food deprivation (statistically significant main effect for groups, \( F(2,18) = 25.43, P < 0.05 \)). Three out of four of the samples obtained from the FD animals fell below the limit of detectability of the assay (0.5 ng/ml) and were assigned a value of 0.5. Thus the values shown here may overestimate leptin levels in these animals. Because this manipulation also truncates the variability in this group, however, these results should be regarded as preliminary.

**DISCUSSION**

The present study demonstrates that 48 h of food deprivation at the end of the second week of lactation suppresses reproductive function in lactating rats. These data are consistent with those of previous studies in rats and hamsters demonstrating that a similar food deprivation schedule disrupts estrous cyclicity (7, 16, 24). Interestingly, the \( \sim 7 \)-day increase in length of lactational diestrus observed in the current study is
similar to that of dams restricted to 50% of ad libitum food intake for the first 2 wk of lactation (29, 30).

Giving FD females AL litters to nurse after the food deprivation period decreased the length of lactational infertility, suggesting that, with this paradigm, the nutritional history of the dam and of the litter that she suckles after the food deprivation period both contribute to the effects of food availability on reproductive function. These data contrast with previous results showing that the length of lactational infertility of chronically food-restricted dams is unaffected by the nutritional history of the pups (30). Thus it appears that both acute and chronic shortages of food extend the length of lactational infertility but that they do so by somewhat different mechanisms.

Leptin administration during the fasting period eliminated the effect of food deprivation on length of lactational diestrus without changing maternal or litter weight loss. There was, however, a slight retardation of maternal weight gain during the postfasting period in females in the FD + leptin group. These data are consistent with those of Ahima et al. (2) demonstrating that leptin administration reversed the effects of food deprivation on estrous cyclicity in mice. Unlike these results, however, no differences in food intake between leptin-treated and vehicle-treated FD groups were observed in the postfasting phase in the current study, and both groups ate less than the AL animals.

Our preliminary data suggest that circulating leptin levels in lactating animals were reduced by food deprivation. Thus the demonstration here that leptin administration is able to eliminate the effects of food deprivation on the length of lactational infertility suggests that circulating leptin levels may play a physiological role in this phenomenon. These data join a growing body of evidence to suggest that leptin influences reproductive function in a variety of situations. Leptin treatment has been used to restore fertility in genetically obese (ob/ob) mice (3), to induce the early onset of puberty in lean female mice (9), and to reduce the effects of food deprivation on estrous cyclicity in mice (2).

What role if any the low level of circulating leptin observed in AL lactating rats plays in the suppression of reproductive function at this time remains to be determined. Brogan et al. (5) reported that lactating rats on day 10 pp had lower levels of circulating leptin than nonlactating rats, and here we obtained similar results when we compared nonlactating rats with dams on day 15 pp. These data suggest that AL lactating animals have chronically low leptin levels. In the current study we found that leptin administration to AL lactating rats on days 13 and 14 pp had only a small, statistically nonsignificant effect on length of lactational infertility. A more prolonged period of leptin administration may be necessary to affect reproductive function in AL lactating rats.

The pattern of leptin administration used in this experiment was sufficient to reduce food intake in AL lactating rats and to suppress litter growth. The pathway through which this latter effect was achieved is not clear. Litter growth could be affected through a reduction in milk production consequent on the decrease in food intake of the dams or through a direct effect on the mammary gland or maternal behavior. Interestingly, recent data suggest that leptin is detectable in human breast milk and that the levels in milk reflect the levels in the maternal circulation (13). Thus the reduction in litter growth produced by leptin administration might also reflect a direct effect of leptin on the pups.

Although the ability of leptin to modulate the reproductive axis is clear (2, 3, 9), the mechanism through which the effects of leptin administration are produced is less well defined. Leptin receptors have been localized in many endocrine and neuroendocrine tissues (32). Furthermore, the results of in vitro studies suggest that leptin influences ovarian function, increases the release of LH and follicle-stimulating hormone from the pituitary, and stimulates GnRH release from arcuate nucleus/median eminence hypothalamic explants (31).

It is also possible that leptin acts indirectly to modulate reproductive function. Leptin decreases neuropeptide Y (NPY) synthesis in the arcuate nucleus in both satiated and FD animals and NPY itself has been shown to affect reproductive function (17). For example, the prolonged period of lactational diestrus observed in food-restricted dams is mediated by a prolonged suppression of LH levels that persists for at least 5 days after the dams are refed (28). These low levels of LH are thought to be accompanied by suppression of GnRH release from the hypothalamus (28) and to be related to food restriction-induced increases in NPY release (1). It has also been suggested that leptin administration changes levels of circulating glucose and free fatty acids (21, 22) and modulates glucose metabolism (14). Such changes could affect reproductive function by changing the activity of metabolic fuel receptors.

Perspectives

The suppression of reproductive function during lactation and its enhancement by food shortages is seen in many mammalian species, but the mechanism(s) underlying this phenomenon are not clear. The results of these experiments show that, in rats, a 48-h fast on days 13 and 14 pp is sufficient to prolong the length of lactational diestrus. Both the nutritional status of the dam and her litter contribute to this effect. Acute food deprivation was more effective in prolonging lactational infertility if the dam continued to nurse previously undernourished pups. These data contrast with results obtained using a chronic food restriction manipulation (30). Thus the contribution of pup condition to this phenomenon varies with the food restriction regimen.

Leptin administration during the period of food deprivation is sufficient to completely prevent the prolongation of lactational infertility. Hence increasing leptin levels in FD dams overrides the effect of nursing previously underfed pups as well as the effects of food deprivation on the dam herself. Furthermore, the finding that FD lactating rats have lower plasma leptin
levels than AL dams suggests that the effect of leptin in this paradigm is physiologically relevant. AL dams have lower levels of circulating leptin than nonlactating females, but the pattern of leptin administration used here was not sufficient to shorten lactational infertility in these animals, although it did reduce food intake and litter growth. Thus the question of what role, if any, low circulating leptin levels may play in the suppression of fertility during lactation remains to be elucidated.

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