Intracerebroventricular Leptin Administration Reduces Food Intake in Pregnant and Lactating Mice[1]

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Leptin acts within the hypothalamus to diminish food intake. During pregnancy and lactation, both circulating leptin concentrations and food intake are elevated, suggesting an ineffectiveness of leptin to reduce food intake in these mice. Thus, this study tested the ability of intracerebroventricular (ICV) leptin administration to alter food intake during pregnancy and lactation. Mice during the first, second, and third trimesters of pregnancy, lactating mice on postpartum Day 7, and age-matched female mice were used. Plasma leptin concentrations averaged 2.9 ± 0.3 ng/ml in control mice, increased steadily as pregnancy progressed (3.4 ± 0.7, 29.8 ± 4.5, and 40.5 ± 0.7 ng/ml during the first, second, and third trimesters, respectively), and remained elevated on Day 7 postpartum (26.4 ± 7.8 ng/ml). Mice were food deprived for 4 h, injected ICV with vehicle or leptin (1 μg), and food intake was subsequently measured hourly for 3 hr, and after 24 hr. Vehicle-treated pregnant mice consumed marginally more food than cycling control mice, whereas nursing dams ate two to three times as much food as controls. As expected, ICV leptin administration reduced 24-hr food intake of control mice by 2 g, or ~50%. ICV-administered leptin was as effective in reducing food intake of pregnant and lactating mice as observed in control mice. Thus, the elevated circulating leptin concentrations observed in pregnant and nursing mice did not alter the ability of ICV-administered leptin to diminish food intake. High plasma concentrations of leptin-binding proteins observed during pregnancy, and probably during lactation, may limit the amount of endogenous leptin reaching the hypothalamus, and may consequently enable increases in food intake concomitant with elevated plasma leptin during these nutritionally demanding periods. [Exp Biol Med Vol. 227(8):616–619, 2002]

Key words: leptin; pregnancy; lactation; food intake; mice

It is now well established that leptin, a polypeptide hormone released primarily from adipose tissue into the circulation, acts within the hypothalamus to regulate body weight by diminishing food intake and elevating metabolic rates (1–3). In the hypothalamus, leptin binds to a receptor to evoke its effects on food intake. There are several forms of the leptin receptor, but the two most characterized types in the rodent are a long form of the receptor, which is present abundantly in the hypothalamus, and a short form, which is widely expressed in the brain and in other tissues (4–6). Signal transduction elicited by the coupling of leptin to the long form of the leptin receptor leads to a reduction in food intake (7–10).

During pregnancy and lactation, dietary requirements are enhanced to sustain fetal growth and milk production. Pregnancy stimulates an increase in synthesis of leptin within adipose tissue of rats and mice, as indicated by a 2- to 5-fold increase in leptin mRNA (11–12). Additionally, the placenta synthesizes leptin, although its quantitative contribution to plasma leptin is not clear (11–16). Consistent with increased synthesis of leptin during pregnancy, plasma leptin concentrations are typically elevated during pregnancy (17–19), although there is a report (20) that plasma leptin concentrations in rats do not rise during pregnancy. An elevation in plasma leptin during pregnancy and lactation seems paradoxical in light of increased dietary requirements to nourish the fetus and newborn. These pregnant and lactating mice must develop a physiological leptin resistance to maintain elevated food intake. This resistance to leptin action might occur at the blood-brain barrier where transport of leptin from the blood to the cerebrospinal fluid may be limited, or it might occur within the hypothalamus at leptin receptor or postreceptor signal transduction pathways. Intracerebroventricular (ICV) injections of leptin would bypass the blood-brain barrier, and should provide important clues to help explain why elevated plasma leptin and food intake are able to coexist during pregnancy and lactation. Thus, this study evaluated the effects of ICV-administered leptin on food intake of pregnant and lactating

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mice. Leptin concentrations in plasma of cycling, pregnant, and lactating mice were also measured.

Methods

Animals. Mice were obtained from our colony of C57BL/6J mice. The *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985) and local institutional guidelines were followed for the care and treatment of the mice. They were singly housed under controlled conditions of temperature (25°C) and light (lights on from 0700–1900 hr) and had free access to a nonpurified diet (Teklad Rodent Diet 8640; 22% protein, 5% fat, and 4.5% crude fiber; Harlan, Bartonville, IL). Male mice were introduced into the cages of cycling10- to12-week-old females that had previously given birth once. The presence of sperm in the vaginal smear or the start of continuous diestrous was designated as Day 1 of pregnancy. Pregnant mice were tested during the first (gestational Days 4 and 6), second (gestational Days 9–13; six mice were tested on Days 9 and 10 of gestation, and two mice were tested on Day 13), or the third trimester (gestational Days 17–19). Lactating mice had litters adjusted to six pups per litter at birth, and were used on Day 7 postpartum. Age-matched female mice during diestrous were used as controls.

Experimental Design. Mice were food deprived for 4 hr, followed by an ICV injection of 2 μl of artificial cerebrospinal fluid ± 1 μg of leptin. This dose of leptin was used because it maximally reduced food intake in mice in previous studies in our laboratory (3). Mice were presented with preweighed pellets of nonpurified diet, and food intake was measured hourly for 3 hr, and 24 hr later. Mice were sacrificed 24 hr after vehicle or leptin administration; blood was collected and plasma separated for leptin determination.

ICV Injection. Mice were lightly anesthetized with ether before ICV injections were made into the lateral ventricle as described earlier (3).

Leptin Source and Measurement. Murine *ob* cDNA was expressed in *Escherichia coli* BL 21 (DE3) and was purified as described before (3). The final preparation was tested for endotoxin and was >98% pure. Leptin was dissolved in phosphate-buffered saline and frozen; aliquots were thawed and diluted with artificial cerebrospinal fluid immediately before use. Leptin was assayed in plasma samples by a radioimmunoassay procedure using a mouse leptin assay kit purchased from Linco Research (St. Charles, MO).

Statistical Analysis. Data are expressed as means ± SEM and were analyzed using SigmaStat (version 2.0; Jandel Scientific, San Rafael, CA). Differences were considered significant at a P value of 0.05 or less.

Results and Discussion

Circulating leptin concentrations increased progressively as pregnancy advanced. Mice during the second (n = 8) and third (n = 6) trimesters of pregnancy, and lactating mice (n = 7) had ~10 fold higher leptin concentrations than control cycling mice (n = 11; Fig. 1). These findings are

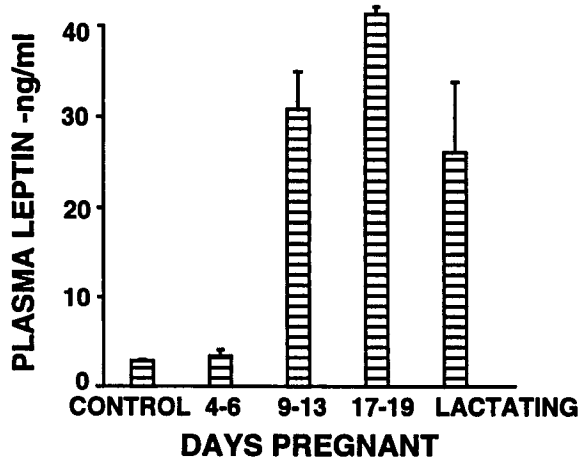

Figure 1. Plasma concentrations of leptin in diestrous mice (n = 11), mice on Days 4–6 (n = 6), 9–13 (n = 8), or 17–19 (n = 6) of pregnancy, and lactating mice on Day 7 postpartum (n = 7). Data are expressed as means ± SEM with the number of mice per group indicated in the parentheses above.

consistent with reports demonstrating that rodents, nonhuman primates, and humans display elevated plasma leptin during pregnancy (12, 17, 21–23). In humans, maternal plasma leptin concentrations were elevated severalfold during pregnancy (22, 24). Likewise, in pregnant baboons, serum leptin concentrations between 60 and 160 days of gestation were 63–157 ng/ml compared with 1.4 ng/ml in nonpregnant animals (25). The physiological significance of elevated plasma leptin during pregnancy is unclear and remains speculative. Leptin administration is required to initiate pregnancy in Lep^{ob}/Lep^{ob} mice lacking endogenous leptin, but was not needed to maintain pregnancy in these mice, suggesting that leptin is not essential for gestation and parturition (26). However, leptin may regulate maternal nutrition via a leptin resistant state and may modulate body composition of adult offspring (27).

As expected, a single ICV injection of leptin (1 μg) diminished food intake of control mice by ~30% within 3 hr, and by 2 g or ~50% within 24 hr (Fig. 2). Daily food intakes of pregnant mice were not elevated during the first trimester (Days 4–6). ICV administration of leptin into these mice reduced 24-hr food intake by ~30%. By 9–13 days of pregnancy, mice were consuming ~35% more food than control mice. ICV leptin injection into these mice decreased 24-hr food intake by 2 g, or ~43%. Likewise, mice in the last trimester of pregnancy consumed about 20% more food than control mice, and ICV leptin curtailed 24-hr food intake of these 17- to 19-day pregnant mice by 3 g, or ~65% (Fig. 2). Lactating mice consumed twice as much food as age- matched controls. Even in these mice, ICV leptin effectively reduced 24-hr food intakes by 4 g or ~50% (Fig. 2). These data are the first to establish that the hypothalamic leptin-induced anorexia is as great in hyperleptinemic pregnant and lactating mice as in control mice. ICV administration of leptin (1 μg) reduced the grams of food consumed (–2 to –4 g) within 24 hr and the percentage of

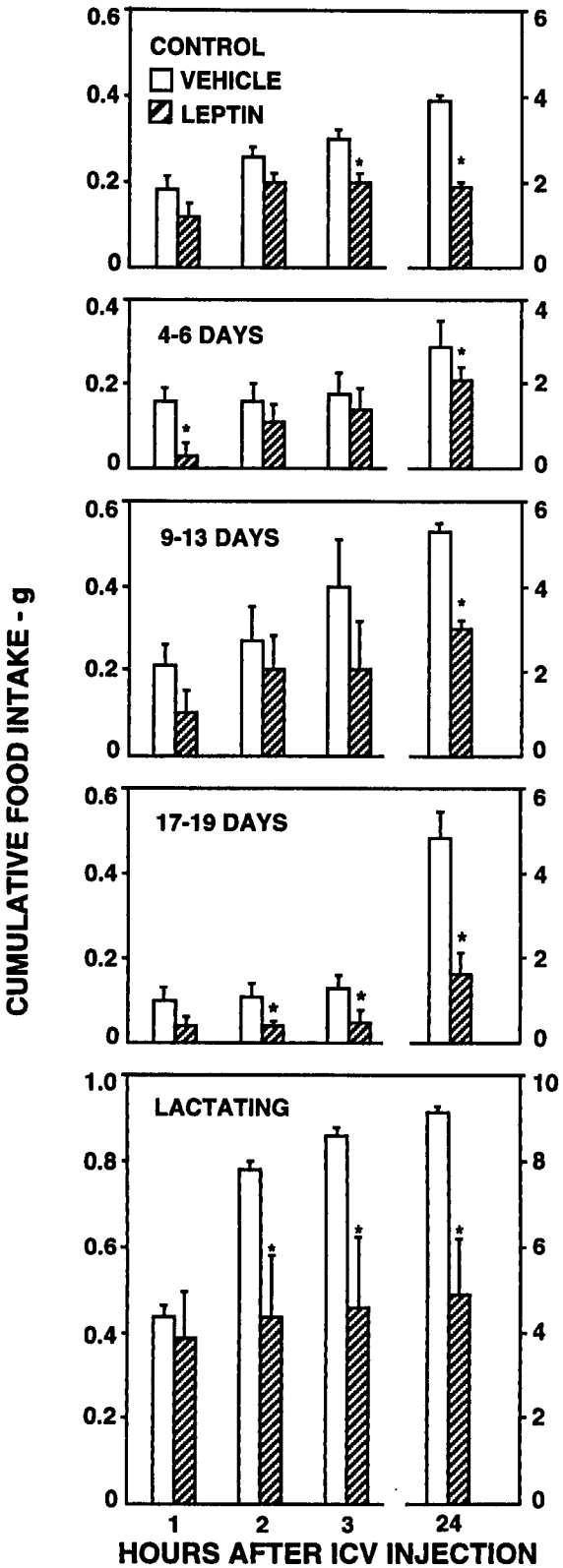

Figure 2. Cumulative food intake of adult cycling female mice (22.5 ± 0.3 g), mice during Days 4–6 (24.3 ± 0.9 g), 9–13 (29.7 ± 1.4 g), or 17–19 (35.6 ± 1.6) of pregnant or lactating mice (26.02 ± 1.2 g) on Day 7 postpartum after a single ICV injection of vehicle or 1 μg of leptin. Body weights are in parentheses. Data are expressed as means ± SEM with six to 11 mice per group. ANOVA, followed by the least significant difference (LSD) test for *post hoc* comparisons, was used for statistical analyses. Asterisks indicate significant differences ($P < 0.05$) between vehicle and leptin-treated mice.

food consumed (–43% to –65%) as effectively in pregnant and lactating mice as in control mice (–2 g or ~50%). Although these results support the conclusion that the responsiveness of the hypothalamic system in hyperleptinemic pregnant and lactating mice is as great as in control mice, it is possible that these mice have impaired hypothalamic sensitivity to leptin. A leptin dose-response test would be needed to assess this possibility. Leptin-treated lactating mice still consumed more food in 24 hr than leptin-treated control mice (Fig. 2). Additional factors, including neuropeptide Y and agouti-related protein, are clearly relevant in modulating food intake during pregnancy and lactation (28).

Circulating leptin normally crosses the blood-brain barrier by carrier-mediated transport (29–30) to bind hypothalamic leptin receptors (31–32) to reduce food intake. The hyperleptinemia in pregnant and lactating mice, coupled with anorexia following ICV administration of leptin, provides support for impaired transport of leptin from blood to the cerebrospinal fluid in these mice. Some obese humans have been reported to have elevated plasma leptin and low cerebrospinal fluid concentrations of leptin, supporting the hypothesis of regulated transport of leptin from the blood to the cerebrospinal fluid (30, 33–34).

Pregnant mice have high concentrations of leptin-binding protein, the soluble form of the receptor, in their plasma (18). This secreted form of the leptin receptor is encoded by the OB-Re isoform and is produced by the placenta (18). The increase in bound leptin may reduce leptin clearance via the kidneys and thereby contribute to elevated circulating concentrations. The bound leptin may also be less available for transport to the central nervous system and signaling through hypothalamic leptin receptors. How and why leptin and the plasma leptin binding protein are regulated during pregnancy and lactation remains unclear.

The concomitant elevations in plasma leptin and food intake characteristic of pregnancy also occur in rodents switched from a high carbohydrate diet to an obesity-inducing, high-fat diet. These high-fat-fed animals do not suppress food intake as effectively after peripheral administration of leptin as observed in animals fed high carbohydrate diets (35, 36). The inability of leptin to reduce food intake develops relatively rapidly (i.e., within hours to a few days [36]). Central administration of leptin to animals fed high-fat diets effectively lowers food intake in some studies (35), but not others (37, 38). One study suggests that insensitivity to centrally administered leptin evolves over a period of weeks after rats are switched to a high-fat diet (37). It is probable that leptin resistance has multiple origins. Pregnancy and lactation, and at least some forms of diet-induced obesity, induce a peripheral-based resistance to leptin that may be linked to the appearance of plasma leptin binding proteins (18).

It seems likely that leptin participates in a broad range of endocrine functions in addition to its well-described role in modulating food intake. The potential physiological func-

tion of leptin in pregnant and lactating dams, as well as in the developing fetus, needs further study.

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