Leptin Accelerates the Onset of Puberty in Normal Female Mice

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Abstract

The fat-derived hormone, leptin, is proposed to serve as an adipostatic signal to the brain to reduce food intake and body weight. In addition to its effects on body weight, chronic leptin treatment restores puberty and fertility to *ob/ob* mice with total leptin deficiency, and acute treatment substantially corrects hypogonadism in mice starved for 2 d without affecting body weight. Leptin may therefore be a critical signal, linking adiposity and reproduction. Since body weight and adiposity appear to play a critical role in the timing of puberty in humans and rodents, and leptin levels rise with increasing adiposity, we studied the effects of once daily injections of recombinant leptin on the onset of puberty in female mice weaned on day 21 and fed ad libitum. There was a linear increase in body weight during the study period, which was not altered by the dose of leptin used. Mice injected with leptin had an earlier onset of three classic pubertal parameters (i.e., vaginal opening, estrus, and cycling) compared with saline-injected controls. Leptin is the first peripheral molecule demonstrated to accelerate the maturation of the reproductive axis in normal rodents. We propose that leptin is the signal that informs the brain that energy stores are sufficient to support the high energy demands of reproduction, and may be a major determinant of the timing of puberty. (*J. Clin. Invest.* 1997. 99:391–395.)
Key words: ob protein • vaginal opening • estrus • cycling

Introduction

Puberty is the final stage of maturation of the hypothalamic-pituitary-gonadal axis, culminating in an adult phenotype (1), and is marked by changes in circulating gonadotropins and increased levels of sex steroids (2, 3). The increased gonadotropin secretion that promotes gonadal development during puberty is itself driven by increased activity of hypothalamic neurons producing gonadotropin releasing hormone (GnRH)[1] (3, 4). Although the GnRH neuron is subject to influence by a number of factors (4), it is unclear whether puberty results from the presence of an activator or the withdrawal of an inhibitor of GnRH neuronal activity (1, 2). The factors that act on the GnRH neuron to initiate puberty have not been identified. Nutrition has a well recognized influence over pubertal development (5–7), and the attainment of a critical body weight and adipose tissue mass have been proposed to determine the onset of puberty (5, 8–10). In support of this idea, body weight in girls is a better a predictor of the timing of puberty than is age (8), and vaginal opening and estrus occur earlier in rats as a function of increasing weight and fat mass (5, 11). Likewise, food deprivation can delay or prevent puberty in humans and other mammals (6). Although such studies have suggested that a signal related to energy stores or adipose tissue mass may be involved in the timing of puberty (9, 10), the existence of a signal that is related to energy stores and is capable of affecting the timing of puberty has yet to be demonstrated.

The recent discovery of leptin, the product of the obese (*ob*) gene (12), has improved our understanding of the relationship between adipose tissue and energy homeostasis (13–19). Leptin is produced by adipose cells, and a rising level of leptin as triglyceride stores increase is proposed to serve as a negative feedback signal to the brain, resulting in decreased food intake, increased energy expenditure, and resistance to obesity (13–15). In addition to this role, which has been proposed to be its primary function (12–15), circulating leptin also appears to play an important role in the neuroendocrine axis (20), including the regulation of reproduction (21–23). Consistent with this hypothesis, *ob/ob* mice with total deficiency of leptin exhibit infertility (21, 22), in addition to hyperphagia and morbid obesity. Chronic leptin treatment not only reduces food intake and weight in *ob/ob* mice, but also restores puberty and fertility (21, 22). Although this result in *ob/ob* mice reveals the ability of chronic leptin repletion to reverse the adverse consequences of total, life-long deficiency of leptin, it does not define a physiologic role of leptin in the regulation of the reproductive axis. Starvation is a physiologic state in which leptin levels fall (17, 19), and we recently have shown that the prolongation of diestrus induced by 48 hours of starvation is substantially prevented by leptin treatment (20). Thus, in sexually mature mice, leptin is a key link between nutrition and the reproductive axis. Here, we substantially extend these findings

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1. *Abbreviations used in this paper:* GnRH, gonadotropin releasing hormone; VC, vaginal cycling; VE, vaginal estrus; VO, vaginal opening.

by showing that leptin treatment of normal, prepubertal female mice accelerates the onset of puberty as determined by vaginal opening, estrus, and cyclicity. Importantly, the effect of leptin on puberty occurs in the absence of any effect on body weight. Leptin, a critical signal linking energy stores to the reproductive axis, may therefore be a primary factor determining the timing of puberty.

Methods

Female C57BL mice (The Jackson Laboratories, Bar Harbor, ME) were weaned at 21 d, and housed in groups of three in metal shoe box cages with constant environmental conditions at 22°C and 12-h light (0600–1800) and dark (1800–0600) cycles. The procedures were approved by the Beth Israel Hospital and Harvard Medical School Institutional Animal Care and Use Committee. The mice were allowed ad libitum access to chow and water, and bedding was changed weekly. No males were housed on the same rack. Recombinant mouse leptin was provided by Eli Lilly and Co. (Indianapolis, IN), and radioimmunoassay kits for leptin and estradiol were obtained from Linco Research (St. Charles, MO) and ICN Biomedicals Inc. (Costa Mesa, CA), respectively.

After determining initial weights, the mice were divided into two groups (n = 15). One group was injected daily with leptin, 2 μg per gram body wt in 100 μl saline i.p. and the other with saline alone at 1000–1100 hours during the light cycle. The dose of leptin was adjusted daily according to body weight. The mice were weighed and inspected daily for vaginal opening by two independent observers. Vaginal smears from mice with vaginal opening were examined daily, and the onset of vaginal estrus and cycling was determined as described previously (23). To determine the relation between body weight, serum leptin, and estradiol during pubertal development, groups of female mice, housed and fed as above, were killed by rapid carbon dioxide inhalation, between 1000 and 1200 hours during the light cycle on days 22, 24, and 26 (before the onset of vaginal opening). Subsequently, groups of mice were killed on the day of vaginal opening and matched with controls without vaginal opening. Blood was obtained for measurement of leptin and estradiol. Data were analyzed by ANOVA and Fisher PSLD.

Results

Fig. 1 *A* illustrates the growth curves for leptin- and saline-treated mice during pubertal development. Both groups displayed a linear increase in body weight after weaning, and the dose of leptin had no significant effect on body weight. Despite the normal rate of growth, the onset of puberty was earlier in leptin-treated compared with saline-treated controls, as determined by vaginal opening, estrus, and cycling (Fig. 1 *B*). Vaginal opening (VO) occurred at 30.3±0.4 days in saline-treated mice compared with 29.2±0.3 d in leptin-treated mice ($P < 0.05$). The median age for VO was 31 d in saline-treated mice and 29 d in leptin-treated mice. Vaginal estrus (VE) was detected in 11 of 15 leptin-treated mice by day 35. In contrast, only 3 of 15 saline-treated mice had VE by day 35. The mean age for VE was 34.4±0.6 d in leptin-treated mice compared with 37.2±0.6 d in saline-treated mice ($P < 0.05$). Vaginal cycling (VC) was detected in only 1 of 15 saline-treated mice by day 38, in contrast with 8 of 15 leptin-treated mice. The mean age for VC was 38.2±0.7 d in leptin-treated compared with 40.7±0.4 d in saline-treated mice ($P < 0.01$).

Fig. 2 illustrates the relation between body weight, and serum leptin and estradiol during pubertal development. There was a linear increase in body weight from day 22 to 34 (Fig. 2 *A*). Serum leptin concentration was 3.80±0.12 ng/ml on day 22, and was not significantly altered after that (Fig. 2 *B*). There was no difference between leptin concentrations in mice with no vaginal opening and those with vaginal opening ($P > 0.05$). Serum estradiol concentration was unaltered until day 26, but increased after that (Fig. 2 *C*). On day 28, estradiol concentration was 25% lower in mice with vaginal opening (Fig. 2 *C*).

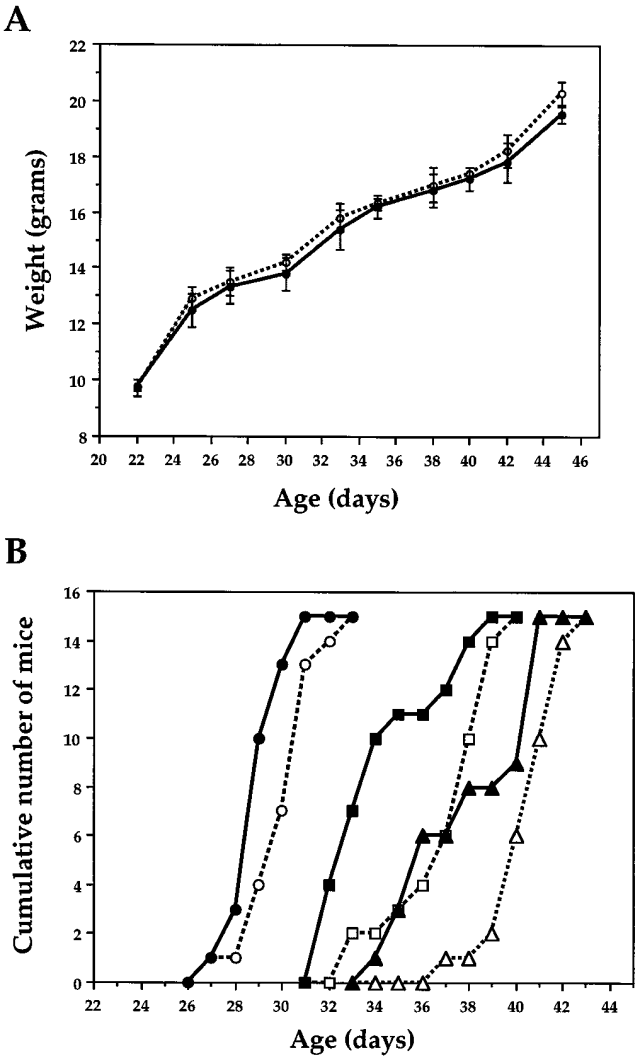

Figure 1. (*A*) Growth curves of female C57BL mice injected with leptin or saline. The mice were weaned at 21 d, housed in groups of three under 12 h light (0600–1800) and dark (1800–0600) cycles, and allowed ad libitum access to chow and water. They were injected once daily at 1000–1100 hours with recombinant mouse leptin, 2 μg/g body wt or saline vehicle, 100 μl i.p., until day 43. Data are means±SEM, n = 15 per group. ○, Saline; ●, leptin. (*B*) Onset of puberty in leptin- and saline-treated female mice. ○, VO salin; ●, VO leptin; □, VE saline; ■, VE leptin; △, VC saline; ▲, VC leptin.

Discussion

The timing of puberty is crucial to the regulation of population growth and density (24, 25), and involves a complex interaction between genetic and environmental factors, such as nutrition and housing (24). A relationship between energy homeostasis and reproduction is well known (6, 24). Nutrition affects

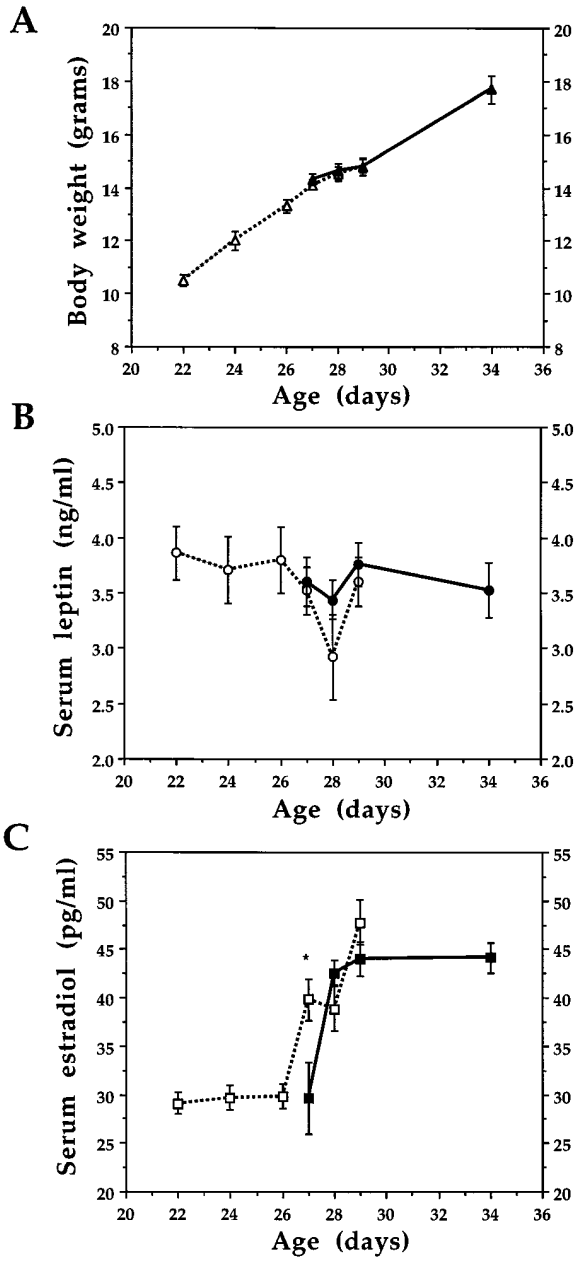

Figure 2. (*A*) Growth curve, (*B*) serum leptin, and (*C*) serum estradiol concentration of female C57BL mice during pubertal development. −VO, before vaginal opening; +VO, vaginal opening. Data are means±SEM, n = 5–16 per group. $^*P < 0.05$ compared with mice with vaginal opening by ANOVA and Fisher FSLD. Open symbols, −VO; closed symbols, +VO.

puberty, cyclicity, pregnancy, and lactation (6), and is a critical determinant of population growth (24, 25). In humans, states of negative energy balance (e.g., starvation) uncontrolled diabetes, and high intensity exercise are associated with disruption of cyclicity (26–28). Fecundity is better correlated with body weight and adiposity than with age (6), and the trend towards puberty occurring at younger ages in developed countries during this century may be determined by improved nutrition and adiposity in children (8). Vaginal opening and estrus in rodents are also influenced by adiposity (11). Rats fed a high fat diet in which fat was substituted isocalorically for carbohydrate had earlier onset of estrus than low fat rats (11). Carcass analysis showed that despite weighing less, the high fat rats were fatter than low fat rats at first estrus (11).

The existence of metabolic signals capable of regulating the reproductive axis has been postulated (5, 11). Earlier studies suggested that fat-derived signals (e.g., fatty acids and estrogen) (11, 29, 30) could link adiposity and reproduction, while Wade et al. (31) have proposed instead that glucose is the metabolic signal involved in the central regulation of reproduction. Since concentrations of the fat-derived hormone, leptin, correlate with adipose tissue mass in the fed state (16–18) and leptin levels fall with starvation (17, 19), leptin could provide the relevant information to the brain. This speculation is supported by two lines of experimentation. Thus, leptin treatment reverses hypogonadism in two distinct states: totally leptin-deficient *ob/ob* mice (21, 22) and normal mice with partial leptin deficiency due to starvation (20). We therefore hypothesized that leptin may be a critical determinant of the timing of puberty. In this study we report that treatment of ad lib fed female mice with a dose of leptin that does not alter body weight significantly, results in an earlier onset of vaginal opening, estrus, and cycling. The ability of exogenous leptin to accelerate puberty without affecting body weight in normal mice suggests that leptin may be the factor that normally links body weight and energy stores to the timing of puberty. How do these findings relate to the actions of leptin to regulate body weight? Whereas totally leptin-deficient *ob/ob* mice respond to low doses of leptin with marked weight loss (13–15), treatment of normal mice with similar doses of leptin causes less weight loss (13–15). Our observation that daily treatment with recombinant leptin accelerates puberty in normal mice at doses that do not change body weight suggests that actions of leptin to regulate neuroendocrine and reproductive function in normal mice are not secondary to effects on energy balance.

Puberty may be thought of as the period when reproduction is first possible, and in rodents may manifest as the first display of lordosis. However, since the latter behavior is often not observed in laboratory animals, other parameters have been used to characterize the onset and progression of puberty (23, 32). Of the three parameters used in this study, vaginal opening correlates less with ovulation than do vaginal estrus and cyclicity (32). Vaginal opening and estrus have been correlated with changes in estradiol levels during pubertal development in mice (33). Estradiol has been reported to peak one day before vaginal opening, decrease thereafter and increase to another peak during vaginal estrus (33). We determined the relationship between body weight, leptin, and estradiol during pubertal development in a population of female mice in an attempt to detect a relationship between the onset of vaginal opening and the level of leptin in the blood. Although body weight rose linearly between days 22 and 34, the mean leptin level remained constant across this period. Although we were unable to detect a difference in mean leptin levels in mice of the same age with and without VO, in preliminary studies leptin levels are higher during the early postnatal period in female mice. Moreover, data from a longitudinal study of puberty in boys suggests that a rise in serum leptin occurs 3–6 mo before the initial rise in serum testosterone (Mantzoros, C., J.S. Flier and A.S. Rogol, unpublished data). Thus a rise in leptin precedes and may be causally linked to the onset of puberty.

This study did not directly address the cellular and bio-

chemical targets through which leptin accelerates puberty. The actions of leptin to regulate energy balance appear to be primarily through actions in the brain (13, 33–36), in particular the hypothalamus (35, 36). Since the signaling form of the leptin receptor has been reported to be present in gonadal tissue (31), it is possible that leptin might exert an action on the gonad, although no such action has yet been described. With leptin treatment of *ob/ob* (22) and starved normal mice (20), levels of gonadotropins are increased along with sex steroids, demonstrating a capacity for central action on the reproductive axis. We therefore speculate that the changes reported here may result from central, rather than peripheral actions of leptin on the reproductive axis. Although we have not determined the mediators of leptin effects on the timing of puberty, the GnRH neuron is a potential target (1, 2). Changes in the frequency and magnitude of GnRH pulses herald the onset of puberty (3, 4), and it is plausible that leptin exerts direct actions on the GnRH neuron, such as modifying its inherent pulsatility (37, 38) or capacity to express GnRH, or through intermediation of another factor. Neurotransmitters such as glutamate, GABA, and NPY have been proposed as potential regulators of GnRH activity during puberty (4, 39–41). Leptin suppresses hypothalamic NPY gene expression (20, 36) and release (42), and could potentially modulate levels of NPY and other neurotransmitters during puberty, leading to activation of the GnRH neuron. Whatever the cellular target in the hypothalamus, it is interesting that leptin has a diurnal pattern of secretion, with increased levels during sleep in humans (43) and the dark cycle in rodents (20). We speculate that increased levels of leptin during the prepubertal period could mediate the nocturnal enhancement of gonadotropin secretion of puberty (3).

In summary, we have shown that puberty is accelerated in normal female mice injected daily with increasing doses of recombinant leptin. Although we have not determined the role of endogenous leptin in the physiology of puberty, we speculate that in addition to serving as a signal linking energy stores in adipose tissue to sites in the brain involved in feeding responses (12–15), leptin may inform hypothalamic sites involved in the coordination of the reproductive axis that sufficient energy stores are present to meet the demands of reproduction. The evolution of a system that links energy stores and use, and triggers adaptive responses to preserve energy homeostasis, is crucial for survival of the species. Since reproduction places a high demand on energy stores and puberty confers reproductive competence, and is an important determinant of population growth and density (24, 25), the evolution of a metabolic signal linking the timing of puberty to energy stores is plausible.

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