Is Leptin a Key Factor which Develops Obesity by Ovariectomy?

KENJI SHIMOMURA, HIROYUKI SHIMIZU, TAKAHUMI TSUCHIYA, YUMIKO ABE*, YUTAKA UEHARA AND MASATOMO MORI

First Department of Internal Medicine and *Department of Gynecology and Obstetrics, Gunma University School of Medicine, Maebashi, Gunma 371–8511, Japan

Abstract. Withdrawal of estrogen by ovariectomy increases adiposity, but decreases the circulating levels of the ob gene product, leptin, which inhibits food intake. The reduction of circulating leptin levels may thus play an important role in the induction of obesity by ovariectomy. To examine this hypothesis, body weight changes by ovariectomy were investigated in leptin-deficient genetically obese (ob/ob) mice with leptin supplement. Prior to the operation, obese (ob/ob) female mice were treated with intraperitoneal administration of recombinant mouse leptin (1.0 μg/g body weight/day) for 8 days. Then, half of the leptin-treated mice and their lean littermates were bilaterally ovariectomized and their body weight changes were observed for 56 days. From 16 days after the operation, a significant increase in body weight by ovariectomy was observed only in lean mice without leptin treatment. From 44 days, a significant body weight gain by ovariectomy was observed in leptin-treated obese mice. Ovariectomy significantly increased retroperitoneal white adipose tissue weight in their lean littermates, but not in leptin-treated obese mice. It was suggested that the reduction of circulating leptin levels may play an important role in the increases of acute phase body weight gain by ovariectomy, but during static phase, the direct effects of estrogen withdrawal may appear independent of leptin-mediated effects.

Key words: Leptin, Ovariectomy, Estrogen, Genetic obesity, ob/ob mice


STUDIES have shown that sex hormones are involved in the regulation of appetite and body weight, and that withdrawal of estrogen increases adiposity in rodents [1, 2]. Our long-term observation for 12 months demonstrated that withdrawal of estrogen by bilateral ovariectomy temporality increases food intake and decreases motor activity, resulting in body weight gain in rats [3]. Estrogen directly modulates ventromedial hypothalamic neuron activities [4] and affects hypothalamic gene expressions of the neuropeptides regulating feeding such as neuropeptide Y and corticotrophin releasing hormone [5]. While the issue of whether ovariectomy affects thermogenesis through modulating sympathetic activities remains controversial [6, 7]. The direct effects of estrogen on those factors can’t completely explain the mechanism of hyperphagia and subsequent body weight gain by withdrawal of estrogen due to the ovariectomy.

The anorexic peptide, leptin, was found in genetically obese (ob/ob) mice [8]. Leptin inhibits food intake and enhances sympathetic nerve activities in the hypothalamus [9, 10]. However, it has been previously reported that the effect of estrogen on body weight gain is attenuated in Zucker rats [11], which exhibit the missense mutation in the cDNA for the leptin receptor, producing a single amino acid substitution in the extracellular domain of the receptor [12]. We have demonstrated that the circulating leptin levels are lower in ovariectomized rats than in in-
tact animals, and that estrogen supplement increases circulating leptin levels [13]. Intracerebroventricular infusion of leptin reduced food intake in ovariec- tomed ewes, even though food intake did not change in controls [14]. Leptin receptor gene expression was reduced by estradiol administration and increased by ovariectomy [15]. Estrogen appears to be involved in the leptin effect on feeding behaviors. It is supposed that the reduction of circulating leptin levels may play an important role in the induction of obesity by ovariectomy. To examine this hypothesis, the effects of ovariectomy on body weight change should be investigated in genetically leptin-deficient obese (ob/ob) mice which show no changes of circulating leptin levels by ovariectomy. The present study was designed to examine the possible involvement of leptin in body weight change by ovariectomy in leptin-treated ob/ob mice.

**Materials and Methods**

**Animals**

Female genetically obese (ob/ob) mice and their lean littermates, C57BL/6J (+/−) black mice, were obtained from The Jackson Laboratory (Bar Harbor, Maine, U.S.A.) at age of 7 weeks. The animals were housed in a temperature-controlled room, and given free access to laboratory chow pellets and drinking water.

**Operation**

Prior to the operation, genetically obese (ob/ob) female mice were treated with intraperitoneal administration of recombinant mouse leptin (R&D Systems, Inc., Minneapolis, U.S.A., 1.0 μg/g body weight/day) for 8 days. Then, half of leptin-treated mice and their lean littermates were bilaterally ovariectomized under light ether anesthesia and body weight changes were observed for 56 days. Leptin treatment for obese mice continued by the end of the experiment. Their lean littermates were not treated with leptin during whole observation period. At 56 days after the operation, all animals were sacrificed at 12 hours after last leptin injection and blood samples were collected. After centrifugation, serum was frozen until assay. Uterus and right retroperitoneal adipose tissue weight were immediately measured.

**Assay**

Serum estradiol concentrations were measured by the Double Antibody Estradiol radioimmunoassay (RIA) kit (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) after extracting the sample with diethyl ether. β-Estradiol (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as a reference standard. Serum immunoreactive leptin (IRL) concentrations were measured by commercially available RIA kit (Linco Research, Inc., St. Charles, MO, U.S.A.).

**Statistics**

Data are expressed as mean ± SE. The statistical analysis of the mean was performed by the analysis of variance (ANOVA), followed by Duncan’s multiple range test for the comparison of the means.

**Results**

Body weight changes after leptin administration and ovariectomy are presented in Fig. 1 (obese mice) and 2 (lean mice). Prior to the operation, leptin was subcutaneously injected for 8 days and the treatment obviously reduced body weight gain in obese mice. Then, half of the leptin-treated obese mice and their non-leptin-treated lean littermates were bilaterally ovariectomized (day 0). From 16 days after the operation, ovariectomy significantly increased body weight in lean littermates (Fig. 2), but not at all in obese mice (Fig. 1). Thereafter, body weight gradually increased in lean mice, but no significant body weight gain was observed in obese mice for about 1 month after the operation. Finally, body weight gain of ovariectomized, leptin-treated obese mice was significantly higher than in sham-operated, leptin-treated obese mice at 44, 48, and 52 days after ovariectomy.

Figs. 3 and 4 show changes in uterus and retroperitoneal white adipose tissue weight in lean and obese mice. As shown in Fig. 3, leptin treatment significantly increased uterus weight of obese mice to the same levels as their lean littermates. Bilateral ovariectomy significantly decreased uterus weight in
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**Fig. 1.** Changes in body weight of genetically obese (ob/ob) mice after intraperitoneal administration of 1.0 μg/g body weight/day recombinant mouse leptin and bilateral ovariectomy (OVX). Arrow shows the time of ovariectomy or sham-operation. N = 7 (non-leptin treated obese mice), 5 (leptin-treated, sham-operated obese mice), 6 (leptin-treated, ovariectomized obese mice). *: p<0.01 vs. non-leptin treated obese mice; +: p<0.05 vs. leptin-treated, sham-operated obese mice.

**Fig. 2.** Changes in body weight of C57Bl/6J lean black mice after bilateral ovariectomy. N = 9 (sham-operated lean mice), 11 (ovariectomized lean mice). +: p<0.05; *: p<0.01 vs. sham-operated lean mice.
Fig. 3. Changes in uterus weight of genetically obese (ob/ob) and C57B1/6J lean black after intraperitoneal administration of 1.0 μg/g body weight/day recombinant mouse leptin and bilateral ovariectomy. N = 7 (non-leptin treated obese mice), 5 (leptin-treated, sham-operated obese mice), 6 (leptin-treated, ovariectomized obese mice). N = 9 (sham-operated lean mice), 11 (ovariectomized lean mice).

Fig. 4. Changes in retroperitoneal white adipose tissue weight of genetically obese (ob/ob) mice and C57B1/6J lean black mice after intraperitoneal administration of 1.0 μg/g body weight/day recombinant mouse leptin and bilateral ovariectomy. N = 7 (non-leptin treated obese mice), 5 (leptin-treated, sham-operated obese mice), 6 (leptin-treated, ovariectomized obese mice). N = 9 (sham-operated lean mice), 11 (ovariectomized lean mice).

both lean and leptin-treated obese mice. Leptin treatment significantly decreased retroperitoneal adipose tissue weight in obese mice (Fig. 4). Ovariectomy significantly increased retroperitoneal white adipose tissue weight in their lean littermates. In contrast, a significant increase of retroperitoneal white adipose tissue weight was not observed in leptin-treated obese mice.

Leptin treatment significantly increased serum estradiol concentrations in obese mice and bilateral
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ovariectomy decreased serum estradiol levels (non-leptin treated obese mice, 23.02 ± 2.44 pg/ml; leptin-treated, sham-operated obese mice, 41.92 ± 6.12 pg/ml (p<0.01 vs. non-leptin treated obese mice); leptin-treated, ovariectomized obese mice, 28.04 ± 2.80 pg/ml (p<0.05 vs. leptin-treated, sham-operated obese mice)). Serum estradiol concentrations of non-leptin treated obese mice were similar to those of leptin-treated, ovariectomized obese mice. On the other hand, there was no difference in serum IRL concentrations between leptin-treated, sham-operated obese mice (3.46 ± 0.020 ng/ml) and leptin-treated, ovariectomized obese mice (3.00 ± 0.36 ng/ml). This data confirmed that ovariectomy did not affect circulating leptin levels in leptin-deficient obese mice.

Discussion

We have previously demonstrated that circulating leptin levels are significantly lower in ovariectomized female rats than in intact animals, and that estrogen supplement restored the effect of ovariectomy [13]. These findings are compatible with clinical observations in human subjects [13, 16]. It was hypothesized that the reduction of circulating leptin levels may play an important role in the development of obesity by hyperphagia in ovariectomized female animals. In the present study, 1 μg/g body weight/day leptin treatment decreased body weight accompanied by a significant increase of serum estradiol concentrations and uterus weight, indicating an improvement of ovarian function in leptin-deficient obese mice.

Pellymounter et al. have reported that intraperitoneal administration of 1 μg/g body weight/day leptin was sufficient to suppress body weight gain in ob/ob mouse, and that the effect of intraperitoneal administration of leptin on body weight was maximal at 10 μg/g body weight/day [17]. Circulating leptin levels are from 1.5 ng/ml to 4 ng/ml in lean rodents [18]. In leptin resistant New Zealand Obese (NZO) mice, circulating leptin levels are three times higher than lean mice [19]. In this study, serum IRL concentrations of 1 μg/g body weight/day leptin-treated, sham-operated and ovariectomized obese mice were 3.46 ± 0.2 ng/ml and 3.00 ± 0.36 ng/ml, respectively. Therefore, it was supposed that the dose of leptin used in this study was adequate to maintain leptin at physiological concentration in the serum.

In those obese mice treated with 1 μg/g body weight/day leptin, circulating leptin concentrations are fixed in a definite range and bilateral ovariectomy did not affect circulating leptin concentrations. The effect of bilateral ovariectomy on body weight gain was determined in these animals. There was no difference in serum leptin concentrations in leptin-treated, leptin deficient ob/ob mice with or without bilateral ovariectomy. Bilateral ovariectomy did not show a rapid increase in body weight gain by 4 weeks after the operation, compared with their lean littermates which were supposed to show reduced circulating leptin levels by ovariectomy. These results indicate that the reduction of circulating leptin concentrations should be important in a rapid increase of body weight gain within 4 weeks after ovariectomy. But, to definitively conclude that reduced leptin plays a key role in ovariectomy-induced obesity, it is necessary to examine the effect of ovariectomy in obese (ob/ob) mice which received leptin treatment for more than 1 month.

We have reported that the withdrawal of estrogen by ovariectomy rapidly increases body weight accompanied by a temporary increase in food intake for 1 month (acute phase), and thereafter, reduces motor activity, resulting in a steady increase of body weight gain (static phase) [3]. A significant body weight gain by ovariectomy was observed in genetically obese (ob/ob) mice at 44 days after operation. Therefore, the reduction of motor activity may be important in the observed body weight gain in genetically obese (ob/ob) mice, rather than increase of food intake by ovariectomy, although we did not measure motor activity in those mice. The issue of whether the effect of estrogen on motor activity is partially mediated by leptin or not is still unclear in the present study. It has been recently reported that voluntary wheel running decreases leptin mRNA expression and serum leptin concentrations in male Osborne-Mendel and SSB/PI rats [20]. Serum leptin concentrations were reduced in response to exercise training [21]. Therefore, there may be a relation between motor activity and leptin synthesis. However, since the circulating leptin concentrations of leptin-treated, ovariectomized mice which were defective in endogenous leptin secretion were the same as those of leptin-treated, sham-operated mice, the supposed
reduction of motor activity should be due to the loss of direct estrogen action by ovariectomy on the hypothalamus [22, 23].

In summary, the present study suggested that the reduction of circulating leptin may play an important role in the increases of acute phase body weight gain and body fat mass related to hyperphagia by withdrawal of estrogen after bilateral ovariectomy. But during the static phase of ovariectomy-induced obesity, it is possible that the direct effects of estrogen on motor activity may appear independent of leptin-mediated effects.

References

levels are reduced in response to exercise training but not hormone replacement therapy. J Clin Endocrinol Metab 81: 3980–3985.
