Diet-induced Obese Mice Develop Peripheral, but Not Central, Resistance to Leptin

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Abstract

Leptin administration reduces obesity in leptin-deficient *ob/ob* mice; its effects in obese humans, who have high circulating leptin levels, remain to be determined. This longitudinal study was designed to determine whether diet-induced obesity in mice produces resistance to peripheral and/or central leptin treatment. Obesity was induced in two strains of mice by exposure to a 45% fat diet. Serum leptin increased in proportion to body weight ($P < 0.00001$). Whereas C57BL/6 mice initially responded to peripherally administered leptin with a marked decrease in food intake, leptin resistance developed after 16 d on high fat diet; mice on 10% fat diet retained leptin sensitivity. In AKR mice, peripheral leptin significantly decreased food intake in both 10 and 45% fat-fed mice after 16 d of dietary treatment. However, after 56 d, both groups became resistant to peripherally administered leptin. Central administration of leptin to peripherally leptin-resistant AKR mice on 45% fat diet resulted in a robust response to leptin, with a dose-dependent decrease in food intake ($P < 0.00001$) and body weight ($P < 0.0001$) after a single intracerebroventricular infusion. These data demonstrate that, in a diet-induced obesity model, mice exhibit resistance to peripherally administered leptin, while retaining sensitivity to centrally administered leptin. (*J. Clin. Invest.* 1997. 99:385–390.) Key words: leptin resistance • high fat diet • food intake • C57BL/6 • AKR

Introduction

Obesity, which now affects up to 35% of the U.S. adult population, is associated with serious comorbidities, including a high incidence of type II diabetes, cardiovascular disease, osteoarthritis, and an increased risk of many forms of cancer. Several genetic models of obesity have been developed in rodents, including the *ob/ob* mouse, which is characterized by hyperphagia, increased adiposity, decreased thermogenesis, hyperglycemia, and insulin resistance. The *ob* gene was identified by positional cloning and encodes the cytokine protein leptin, which is secreted from adipose tissue and is absent in *ob/ob* mice (1). Administration of recombinant leptin to *ob/ob* mice has been shown to reduce food intake, decrease body weight and adiposity, increase thermogenesis, and ameliorate depressed body temperature (2–4). Whether administering leptin to obese humans will have similar effects is not yet known. To date, no mutations in the genes encoding leptin or the leptin receptor have been detected in humans, indicating that the majority of human obesity cannot be attributed to defects in leptin or its receptor (5, 6). In fact, serum leptin levels are highly correlated with body mass index, and obese humans have been observed to have elevated levels of *ob* mRNA and serum leptin relative to nonobese humans (5, 7–14). Therefore, it has been hypothesized that obese humans may be resistant to the effects of leptin.

The determination of whether exogenous leptin will be effective in the treatment of human obesity awaits the results of clinical trials in obese patients. Meanwhile, we have investigated the effects of leptin administration in a longitudinal study using a murine model in which obesity is induced by exposure to a high fat diet (15). This diet-induced model of obesity may more adequately reflect the physiology of the obese human than does the leptin-deficient *ob/ob* mouse. We have found that, in both C57BL/6 and AKR mouse strains, young lean mice responded to peripherally administered leptin with a decrease in food intake and reduction in body weight. As obesity developed, plasma leptin levels increased and the mice became insensitive to peripherally administered leptin. However, obese AKR mice, which were resistant to peripheral leptin treatment, remained highly responsive to leptin when it was administered centrally. These results suggest that obese humans, who appear to be hyporesponsive to their elevated endogenous leptin, may respond to a central nervous system (CNS)[1]-penetrant leptin analogue.

Methods

Animals and diets. 4-wk-old male C57BL/6 and AKR mice were obtained from Charles River Laboratories (Wilmington, MA) or Jackson Labs (Bar Harbor, ME). Animals were housed individually in a reverse light cycle room (dark cycle from 10:00 to 22:00) and were

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1. *Abbreviations used in this paper:* CNS, central nervous system; icv, intracerebroventricular.

provided ad libitum access to Purina Rodent Chow No. 5001 and water for a minimum of a 1-wk acclimation period before study. Purified diets (Research Diets, Inc., New Brunswick, NJ) were formulated as a modification of Surwit et al. (16) and West et al. (17). The low fat diet (No. D12450) contained 10% kcal as fat and the high fat diet (No. D12451) contained 45% kcal as fat, predominantly in the form of lard. Colonies (n = 100/treatment) of chow-fed, 10% fat-fed, and 45% fat-fed mice of both strains were maintained and body weights were recorded weekly. Groups of mice were removed from these colonies at specific time points to carry out the studies described below.

All studies were conducted in an American Association for Laboratory Animal Care accredited facility following protocols approved by the Schering-Plough Research Institute's Animal Care and Use Committee. The procedures were performed in accordance with the principles and guidelines established by the National Institutes of Health for the care and use of laboratory animals.

Synthesis and purification of human leptin. Recombinant human leptin was expressed and purified from *Escherichia coli* as described elsewhere (1, 18). Leptin preparations used in this study displayed a purity of > 97% as judged by Coomassie blue staining of SDS polyacrylamide gels.

Serum leptin. C57BL/6 and AKR mice of various ages (6–24 wk) maintained on either chow, 10% fat diet, or 45% fat diet were exsanguinated by heart puncture after anesthesia, serum was isolated, and serum leptin concentrations were measured using an RIA kit for mouse leptin (Linco, Inc., St. Louis, MO).

Peripheral leptin studies. After 4 d on either 10% fat diet or 45% fat diet ad libitum, basal food intake at 2, 4, 6, and 24 h after intraperitoneal saline injection was determined in 12 animals of each diet/strain combination. Mice were then divided into two groups (n = 6/group) such that basal food intake and initial body weight did not differ between groups. Mice were administered either saline or 10 mg/kg leptin (i.p.) once daily at the beginning of the dark phase for 3 d. Body weight was measured daily in the last hour of the light cycle. Food consumption was normalized to kilocalories consumed using the conversion factors 3.85 kcal/g and 4.7 kcal/g for 10% fat and 45% fat diets, respectively. The experimental paradigm described above was repeated in naive mice after 16 d of dietary treatment in C57BL/6 mice, and after 16 and 56 d of dietary treatment in AKR mice. In the AKR mouse experiment after 56 d of dietary treatment, leptin doses of 3 and 1 mg/kg (i.p.) were also tested.

Intracerebroventricular (icv) studies. A subset (n = 35) of the 45% fat-fed AKR mice described above were icv cannulated 4–5 wk before the day 56 peripheral leptin study described above was performed. The icv leptin study was conducted 3–4 wk (77–84 d) after the day 56 peripheral leptin study. AKR mice were chronically implanted with a single 26G stainless steel guide cannula (Plastics One, Inc., Roanoke, VA) in the lateral ventricle of the brain under ketamine:xylazine anesthesia with the following coordinates: −0.7 mm relative to bregma, 2.0 mm lateral of midline, and 2.0 mm down (19). Saline or leptin (0.1–10 μg; n = 7–14/dose) was infused in a total volume of 2 μl over the course of 1 min via a 33G internal cannula (Plastics One, Inc.) attached to a BAS Bee Syringe Pump (Bioanalytical Systems, Inc., West Lafayette, IN). The infusion cannula was left in place for an additional minute after the infusion. Food consumption and body weight were measured as described above. The full icv dose–response to leptin was completed in two experiments using the same icv-cannulated mice. Animals were allowed a washout period of 7 d between treatments and were rerandomized between experiments. Saline and the 1 μg dose of leptin were included in both experiments. The food intake values for the saline groups from the two experiments were determined not to be statistically different; therefore, the data from the two experiments were pooled. The data for the saline and 1 μg leptin dose reflect an n = 14; all other groups are n = 7.

Statistical analysis. Statistically significant differences between groups were determined using unpaired Student's t test. The relationship between serum leptin concentrations and body weight was determined by simple linear regression.

Results

Obesity was induced in two strains of mice by exposure to a diet in which 45% of the calories were derived from fat; a parallel group of mice was fed a 10% fat diet. The diets were modifications of diets reported by Surwit et al. (16) and West et al. (17) to induce obesity in a variety of mouse strains. Lard was substituted for corn oil to more adequately mimic the human diet of the Western Hemisphere. Over a period of 51 d, C57BL/6 and AKR mice became 25% ($P < 0.00001$) and 38% ($P < 0.0001$) heavier than control mice from the same strains placed on a 10% fat diet (Fig. 1). The difference in body weight between the high fat and low fat groups became statistically significant between 7 and 14 d of dietary treatment. Serum leptin levels in AKR mice on 45% fat diet were highly correlated with increasing body weight ($y = 1.1x - 22.8$; $r = 0.62$, $P < 0.02$) as shown in Fig. 2. When serum leptin concentrations from chow-fed or 10% fat-fed AKR mice and chow-fed, 10% fat-fed, and 45% fat-fed C57BL/6 mice were included in the data analysis, the regression equation remained the same ($y = 1.1x - 20.0$; $r = 0.81$, $P < 0.00001$), indicating that mouse strain or type of diet did not affect the relationship between serum leptin levels and body weight (Fig. 2).

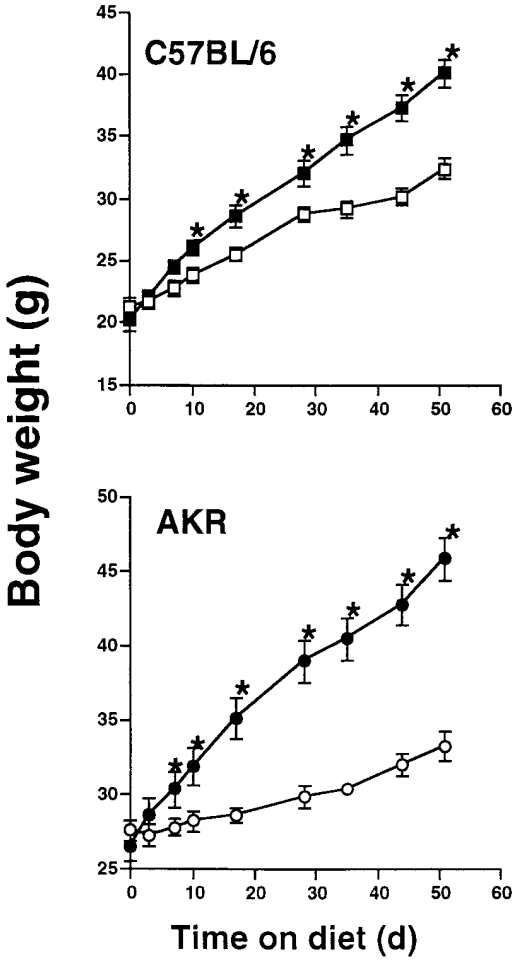

Figure 1. Body weight change in C57BL/6 (*top*) and AKR (*bottom*) mice maintained on 45% fat or 10% fat diets. C57BL/6, 45% (*filled boxes*); C57BL, 10% (*open boxes*); AKR, 45% (*filled circles*); AKR, 10% (*open circles*). Values are mean±SEM (n = 10/group).

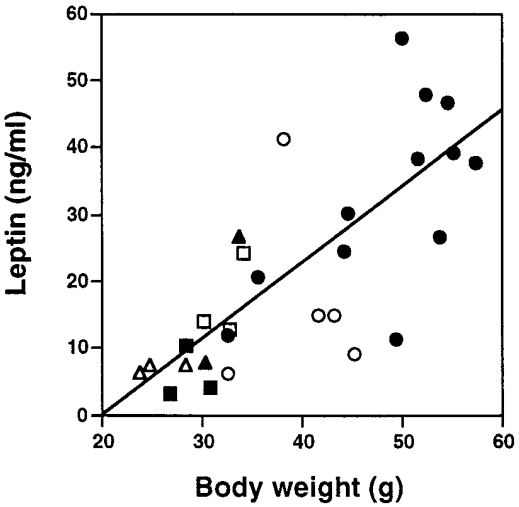

Figure 2. The relationship between serum leptin concentrations and body weight in AKR and C57BL/6 mice of various ages (6–24 wk) fed chow, 10% fat diet, or 45% fat diet. The regression line was derived from the equation: $y = 1.1x - 20.0$ ($r = 0.81$, $P < 0.00001$). Mice: AKR on 45% fat diet (*filled circles*); AKR on chow (*filled boxes*); C57BL/6 on chow (*open triangles*); AKR on 10% fat (*open circles*); C57BL/6 on 10% fat (*filled triangles*); C57BL/6 on 45% (*open boxes*).

After 4 d on either a high fat or low fat diet, C57BL/6 mice responded to intraperitoneal administration of leptin (10 mg/kg) with a marked decrease in food intake (Fig. 3). Cumulative food intake over a 3-d treatment period was inhibited by 20% ($P < 0.0002$) in mice fed a 10% fat diet and by 14% ($P < 0.03$) in mice fed a 45% fat diet for 4 d. After 16 d of dietary treatment, however, only the mice fed a 10% fat diet still responded to intraperitoneal injections of leptin, with an 11% reduction in cumulative food intake ($P < 0.02$, Fig. 3). Leptin did not affect food intake in the mice after 16 d on the 45% fat diet. In all mice that responded to peripheral leptin, there was a small (1.0–2.0 g) but significant ($P < 0.05$) decrease in body weight compared with mice injected with saline. Body weight was not affected in mice which did not show decreased food intake in response to leptin.

Cumulative food intake in AKR mice treated with 10 mg/kg leptin (i.p.) was significantly decreased in both dietary groups after 4 and 16 d of exposure to either a high fat or low fat diet (Fig. 4). AKR mice fed a 10% fat diet showed a 17% reduction in food intake ($P < 0.07$) at 4 d and a 20% reduction ($P < 0.003$) at 16 d in response to leptin. Mice fed a 45% fat diet responded to leptin at 4 d with a 20% reduction in food intake ($P < 0.01$) and showed a 20% reduction in food intake ($P < 0.005$) upon leptin administration after 16 d of dietary treatment. However, after 56 d of dietary treatment, neither the

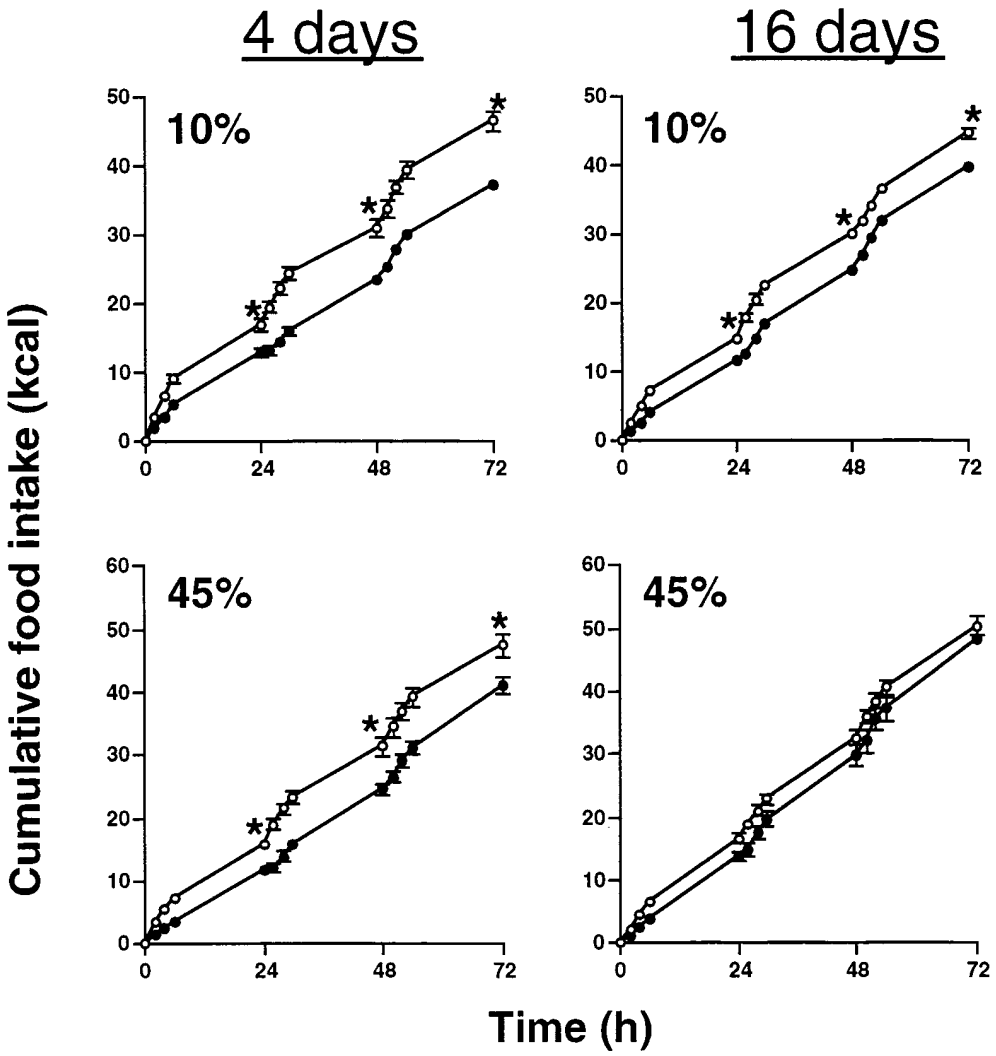

Figure 3. Cumulative food intake (expressed as kilocalories) in 10% (*top*) and 45% (*bottom*) fat-fed C57BL/6 mice after intraperitoneal saline (*open circles*) or leptin (*filled circles*; 10 mg/kg) treatment. The left graphs were obtained after 4 d of dietary treatment; the graphs on the right after 16 d of dietary treatment. The saline or leptin injections were given intraperitoneally at 0, 24, and 48 h. Values are mean±SEM ($n = 6$/group). *Significantly different (at least $P < 0.05$). Asterisks have been placed only at the 24, 48, and 72 h time points which were significantly different. The 2, 4, and 6 h time points after the time points labeled with an asterisk are also significantly different.

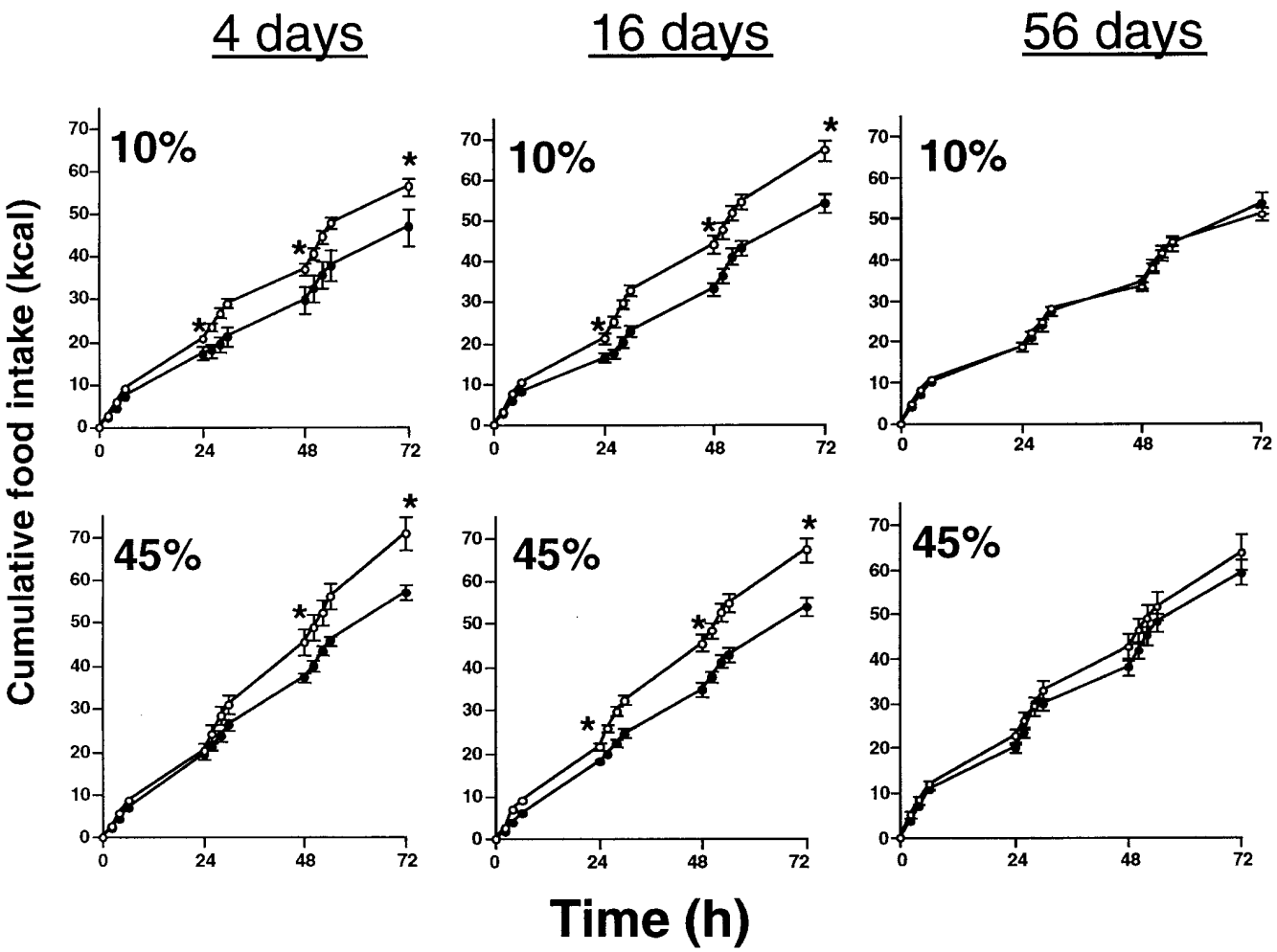

Figure 4. Cumulative food intake (expressed as kilocalories) in 10% (*top*) and 45% (*bottom*) fat-fed AKR mice after intraperitoneal saline (*open circles*) or leptin (*filled circles*; 10 mg/kg) treatment. The left graphs were obtained after 4 d of dietary treatment; the middle graphs after 16 d of dietary treatment; the graphs on the right were obtained after 56 d of dietary treatment. The saline or leptin injections were given intraperitoneally at 0, 24, and 48 h. Values are mean±SEM (n = 6/group). *Significantly different (at least $P < 0.05$). Asterisks have been placed only at the 24, 48, and 72 h time points which were significantly different. The 2, 4, and 6 h time points after the time points labeled with an asterisk are also significantly different but are not labeled with an asterisk.

10% nor 45% fat-fed mice responded to 10 mg/kg leptin (Fig. 4). As was observed in the C57BL/6 mice, there was a small (1.0–2.0 g) but significant ($P < 0.05$) decrease in body weight in AKR mice that responded to peripheral leptin, compared with saline-treated control mice. Body weight was not affected in mice which did not exhibit decreased food intake in response to leptin.

A subset of the AKR mice on the 45% fat diet was implanted with icv cannulas several weeks before the initiation of the studies. Like the uncannulated mice, the icv-cannulated mice were unresponsive to peripherally administered leptin after 56 d of exposure to the high fat diet. The icv-cannulated AKR mice were then continued on a 45% fat diet for an additional 3–4 wk, after which their responsiveness to centrally administered leptin was measured (0.1–10 μg, icv). Leptin, when infused centrally into these peripherally resistant obese mice, caused a dose-responsive decrease in food intake during the 24 h after administration (Fig. 5, *top*). At the highest dose, food intake was inhibited by 72% ($P < 0.00001$) and did not return to baseline levels until 72 h later (data not shown). Body weight also decreased in a dose-dependent matter, with a 7% weight loss ($P < 0.0001$) observed at 24 h after the highest dose (Fig. 5, *bottom*). These results indicate that although AKR mice develop resistance to peripherally administered leptin as their body weight increases, these mice remain highly responsive to leptin delivered directly to the CNS.

Discussion

Although leptin administration has been demonstrated to reduce food intake and ultimately reverse obesity in leptin-deficient *ob/ob* mice (2–4), the relevance of this genetic model to the treatment of human obesity remains to be determined. Obese humans are not leptin deficient. In contrast, serum leptin levels are elevated during obesity, leading to the suggestion that obese humans may be leptin resistant (5, 7–14). Recently, analysis of leptin concentrations in the cerebrospinal fluid has revealed that central leptin levels in obese humans are similar to those of nonobese humans, suggesting that the transport of leptin into the CNS, rather than intrinsic leptin responsiveness,

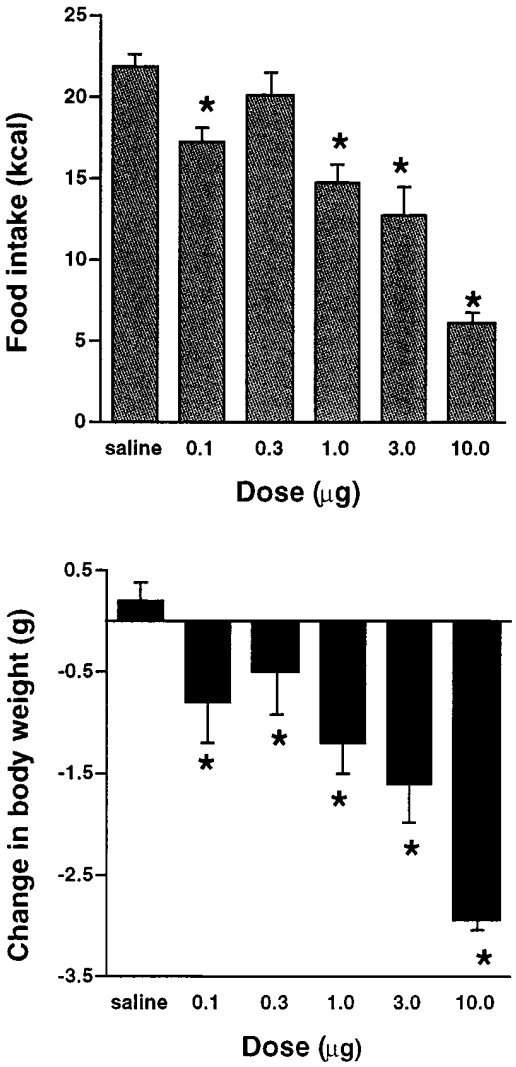

Figure 5. Food intake (*top*) and change in body weight (*bottom*) 24 h after a single icv infusion of saline or leptin at the doses indicated in chronic icv-cannulated AKR mice which were fed a 45% fat diet for 77–84 d. These mice were found previously to be resistant to peripheral leptin treatment (Fig. 4, *bottom right*). Values are mean±SEM (n = 7–14/group). *Significantly different (at least $P < 0.05$).

may be rate-limiting for leptin activity in the obese state (10, 13). In this study, we have addressed this hypothesis in a longitudinal study designed to determine whether the induction of obesity in mice produces resistance to peripheral and/or central leptin treatment.

Obesity was induced in two strains of mice by exposure to a high fat diet for up to 56 d and the efficacy of leptin treatment evaluated at various time points throughout the induction of obesity. AKR mice have been reported to be very sensitive to induction of obesity by high fat diet, possibly due to a defect in thermogenesis (15). C57BL/6 mice have also been reported to gain weight in response to high fat diet, but to a lesser extent than the AKR mice (15). We confirmed these findings in our study: after 51 d, the AKR mice on the high fat diet gained 38% more body weight than the 10% fat-fed controls, while the C57BL/6 mice became 25% heavier than their 10% fat-fed controls. The 45% fat diet thus induced obesity in both strains, with significant increases in body weight compared with the 10% fat-fed mice appearing between 7 and 14 d of dietary treatment. It has been shown previously that the weight gain in these mice is in a number of adipose tissue depots, particularly in the peritoneal cavity (16, 17, 20).

The increase in body weight was accompanied by an increase in serum leptin levels, with a significant correlation between body weight and circulating leptin levels observed in all mice ($P < 0.00001$). Other investigators have also reported increased serum leptin levels in rodent models of obesity, including diet-induced obesity models (9, 21, 22). A similar relationship between leptin levels and body weight has also been observed in a large number of clinical investigations (5, 7–14). Thus, leptin induction in response to diet-induced obesity in mice appears to parallel the response in human obesity.

Peripherally administered leptin inhibited food intake in both mouse strains after 4 d of exposure to either the high fat or low fat diet. However, C57BL/6 mice on the 45% fat diet became resistant to leptin after 16 d of dietary treatment, whereas the 10% fat-fed mice still responded to leptin. In the AKR mice, animals on both diets were responsive to intraperitoneal injections of leptin after 16 d of dietary treatment, but by 56 d both groups were resistant to peripherally administered leptin. It is apparent that peripheral leptin resistance develops in these mice after a given length of dietary treatment and/or a critical body mass or leptin level is reached; the relative contribution of the high fat diet independent of body weight or leptin concentration cannot be assessed at present. Thus, it is possible that C57BL/6 mice on 10% fat diet may also become peripherally resistant to leptin if they reach a critical body weight or adiposity. Strikingly, central administration of leptin to peripherally resistant AKR mice significantly decreased food intake and body weight in a dose-dependent manner. Thus, while these mice are peripherally leptin resistant, they show a robust response to centrally administered leptin.

The average serum leptin concentrations in the AKR mice were ~ 40 ng/ml after 168 d on a 45% fat diet (Fig. 2). Based on a calculated total serum volume of 2 ml (4% body weight), these long-term 45% fat-fed mice would have ~ 80 ng of total circulating leptin. In the leptin administration studies, a relatively high dose of leptin (10 mg/kg, ~ 400 μg/mouse/d, i.p.) was used to evaluate the peripheral responsiveness of mice fed a 45% fat diet for up to 56 d, providing a fairly rigorous evaluation of their leptin resistance. This dose of leptin is up to 25 times higher than that found previously to be very effective in *ob/ob* mice, both in other laboratories (2–4) and in our own laboratory with the same leptin preparation used in the present study (23). This dose is at least 5,000-fold above the endogenous level of leptin in these diet-induced obese mice. In contrast, doses as low as 0.1 μg given directly into the CNS were very active in inhibiting food intake and decreasing body weight in the diet-induced obese mice. Therefore, a peripheral dose of leptin that is 4,000 times greater than the centrally active dose does not overcome the leptin resistance in these mice.

The mechanism for the development of peripheral leptin resistance remains to be determined. One potential mechanism has been demonstrated to occur with homodimeric cytokine receptors, such as the growth hormone receptor (24). High concentrations of the cytokine agonist could promote the formation of inactive monomeric receptors and thereby pre-

vent the formation of active homodimeric receptors. It is not yet known whether the active form of the leptin receptor is a homodimer or a heteromeric complex. Such a mechanism would result in a biphasic dose–response curve to leptin, with lower doses being more efficacious than high levels, such as those that develop in the obese state. To address this hypothesis, we determined that lower doses of leptin (1 and 3 mg/kg, i.p.) were also ineffective in the leptin-resistant AKR mice (data not shown), suggesting that a biphasic dose–response curve for leptin does not appear to account for the peripheral leptin resistance observed in diet-induced obese mice.

Recently, leptin has been shown to enter the brain through a saturable transport system (25). The observation that obese humans do not have elevated cerebrospinal fluid levels of leptin, even though their plasma leptin is elevated in comparison with nonobese humans, has led to the suggestion that this transport system may be saturated or defective in human obesity (10, 13). The results of this study, in which we demonstrate that peripherally leptin-resistant obese mice are responsive to centrally administered leptin, would be consistent with this hypothesis. However, further biochemical experiments will be necessary to directly address the physiological basis for leptin resistance in obese mice and humans.

In summary, we have demonstrated the time-dependent development of peripheral leptin resistance in a murine model of obesity. These mice retain sensitivity to centrally administered leptin. Correlation of this model with human data (7–14) suggests that the leptin resistance hypothesized from the apparent insensitivity of obese humans to their high levels of endogenous leptin may be specific to peripheral leptin. Whether obese humans, unlike the diet-induced obese mice, will respond to administration of high levels of peripheral leptin is not yet known. However, the results of this study suggest that leptin resistance could potentially be overcome by a centrally active leptin analogue.

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