Acute changes in the response to peripheral leptin with alteration in the diet composition

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Acute changes in the response to peripheral leptin with alteration in the diet composition. Am J Physiol Regulatory Integrative Comp Physiol 280: R504–R509, 2001.—Dietary induced obesity in rodents is associated with a resistance to leptin. We have investigated the hypothesis that dietary fat per se alters the feeding response to peripheral leptin in rats that were fed either their habitual high- or low-fat diet or were naïvely exposed to the alternative diet. Osborne-Mendel rats were adapted to either high- or low-fat diet. Food-deprived rats were given either leptin (0.5 mg/kg body wt ip) or saline, after which they were provided with either their familiar diet or the alternative diet. Food intake of rats adapted and tested with the low-fat diet was decreased 4 h after leptin injection, whereas rats adapted and tested with a high-fat diet did not respond to leptin. Leptin was injected again 1 and 5 days after the high-fat diet-adapted rats were switched to the low-fat diet. Leptin reduced the food intake on both days. In contrast, when low-fat diet-adapted rats were switched to a high-fat diet, the leptin inhibitory response was present on day 1 but not observed on day 5. Peripheral injection of leptin increased serum corticosterone level and decreased hypothalamic neuropeptide Y mRNA expression in rats fed the low-fat but not the high-fat diet for 20 days. The data suggest that dietary fat itself, rather than obesity, may induce leptin resistance within a short time of exposure to a high-fat diet.

Food intake; high-fat diet; rats

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tor mRNA expression were also examined at the end of the feeding studies.

MATERIALS AND METHODS

Animals and diet. Fifty male Osborne-Mendel rats from the breeding colony at Pennington Biomedical Center, with a beginning weight of 250 ± 2 g, were used in these experiments. Rats were housed in hanging stainless steel cages in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle (lights on at 0700) and free access to an automatic watering system. They were adapted to either a high-fat diet (56% of energy from fat, 4.78 kcal/g) or a low-fat diet (10% energy from fat, 3.66 kcal/g) for a minimum of 2 wk. The protein content of both diets was identical at 24% of total energy. The composition of these diets has been described previously (19). Food cups were secured in the cages with stainless steel springs, and fresh diet was provided daily. The experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee.

Peptide. The recombinant mouse leptin was a gift from Novartis (Basel, Switzerland) as described elsewhere (22). Leptin was dissolved in saline vehicle (0.9% wt/vol) and was given as a bolus injection on each test day at a dose of 0.5 mg/kg body wt intraperitoneally.

Feeding study. Two separate groups of rats, balanced for body weight at the start of the experiment, were used in this study. The first group was adapted to a high-fat diet for 14 days and then tested for its response to either leptin or saline vehicle on food intake while maintained on this diet. Five days later, these rats were tested again for their response to leptin or vehicle while naïvely given the low-fat diet. The rats were then maintained on the low-fat diet and tested for their leptin response after a further 5 days. A second group of rats was adapted to the low-fat diet for 14 days, tested for its leptin response on this habitual diet, and subsequently tested after naïve exposure to the high-fat diet, as described above. These rats were maintained on the high-fat diet and restested again 5 and 15 days later. The rats were randomized by body weight to leptin or vehicle group for each test. On each test day, rats were food deprived overnight while maintaining free access to water. After being given an intraperitoneal injection of leptin or saline at ~1000, they were returned to their home cages and provided with the diet as described above. Food intake was measured by weighing the food cups 2, 4, 6, and 24 h after leptin or vehicle injections, at each time correcting for all spillage. There was a 4- to 5-day interval between each injection to allow time for rats to recover. Twenty days after the diet switches, the rats fed ad libitum were injected with leptin (0.5 mg/kg body wt) or saline vehicle at 1000, and the food cups were removed. Four hours later, the rats were killed by guillotine. Trunk blood was collected, centrifuged, and the serum was stored at −70°C until assayed for hormones. The hypothalamus was also dissected from each rat, frozen in liquid nitrogen, and stored at −70°C until RNA isolation.

Serum assays. Commercial radioimmunoassays were used for assay of serum leptin and insulin (Linco, St. Charles, MO) and corticosterone (ICN Pharmaceuticals, Costa Mesa, CA).

Isolation of total RNA. Total RNA was extracted from hypothalamic tissue by the modified guanidinium-isothiocyanate method (8) with the use of TRizol Reagent (GIBCO), according to the manufacturer’s instructions.

cDNA probes and Northern blots. The 377-bp NPY plasmid DNA was a gift from Dr. Y. J. Zhou (Pennington Center), and the 5-HT2C receptor plasmid DNA was kindly provided by Dr. D. J. Julius (18). The β-actin DecaProbe was obtained from Ambion (Austin, TX). Twenty micrograms of total hypothalamic RNA were denatured at 65°C for 15 min, electrophoresed on 10 g/l agarose/formaldehyde gels, and transferred to nylon membranes (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA). NPY and 5-HT2C receptor probes were labeled with the use of DECA prime II kit (Ambion) with [32P]dCTP, 3 Ci/mol (111 GBq/mol) (NEN, Boston, MA). Blots were prehybridized with 500 g/l formamide, 0.12 mol/l Na2HPO4−·H2O (pH 7.2), 0.25 mol/l NaCl, and 70 g/l SDS for 1 h at 42°C. Hybridization was performed for 18 h at 42°C in the same buffer containing 1 × 109 counts⋅min−1⋅μg−1 of 32P-labeled probe. Thereafter, membranes were washed at room temperature with 2 × sodium chloride-sodium citrate (SSC)/9 g/l SDS for 15 min, 0.5 × SSC/(g × SDS) for 20 min, and 0.1 × SSC/(g × 1 SDS) for 15 min at 65°C. Membranes were exposed to the PhosphorImager screen overnight, then stripped and rehybridized with β-actin cDNA probe as a control for the gel loading and transfer. Signal intensity was assayed on a PhosphorImage (Molecular Dynamics).

Data analysis. All results are presented as means ± SE. The data were analyzed by ANOVA, and post hoc tests were made using Duncan’s multiple-range test.

RESULTS

Tested diets same as adapted diets. The effects of peripheral leptin injection on the food intakes of rats adapted to either the high-fat or low-fat diet and tested on their habitual diets are shown in Fig. 1. Leptin (0.5 mg/kg ip) treatment reduced food intake in rats eating the low-fat diet (treatment: F1,8 = 13.07, P < 0.0068) (Fig. 1A). This reduction was evident at 6 h after administration of leptin [saline: 34.62 ± 1.65 kcal (9.46 ± 0.45 g) vs. leptin: 23.86 ± 0.45 kcal (6.52 ± 0.56 g); P < 0.05] and lasted up to 24 h compared with the saline group. In contrast, no response was observed to leptin in the rats adapted to the high-fat diet (Fig. 1B).
**LEPTIN RESISTANCE AND HIGH-FAT FEEDING**

High-fat diet-adapted rats tested with low-fat diet. When rats previously adapted to the high-fat diet were naively provided with a low-fat diet immediately after administration of leptin (Fig. 2A), leptin significantly reduced the intake of the low-fat diet \( (F_{1,13} = 4.69, P < 0.049) \). The decreased feeding was observed as early as 4 h \( \text{saline: 21.26 } \pm \text{ 0.03 kcal (5.81 } \pm \text{ 0.83 g) vs. leptin: 13.91 } \pm \text{ 1.02 kcal (3.80 } \pm \text{ 0.24 g; } P < 0.05) \). These rats were subsequently kept on the low-fat diet, and leptin response was retested after 5 days. The suppressive effect of leptin on the intake of the low-fat diet was still evident at this time \( (F_{1,11} = 17.14, P < 0.0016) \) with a 26% reduction compared with controls at 2 h \( \text{saline: 27.05 } \pm \text{ 1.86 kcal (7.39 } \pm \text{ 0.51 g) vs. leptin: 20.06 } \pm \text{ 0.99 kcal (5.48 } \pm \text{ 0.27 g; } P < 0.05) \) and a significant inhibition maintained through the 24-h period.

Low-fat diet-adapted rats tested with high-fat diet. When rats adapted to the low-fat diet were naively presented with the high-fat diet (Fig. 3), leptin significantly suppressed the food intake at all time points from 2 to 24 h \( \text{leptin treatment: } F_{1,9} = 23.46, P < 0.0009) \) \( \text{at 2 h, saline: 27.10 } \pm \text{ 1.67 kcal (5.67 } \pm \text{ 0.35 g) vs. leptin: 14.05 } \pm \text{ 2.53 kcal (2.94 } \pm \text{ 0.53 g; } P < 0.05) \) (Fig. 3A). However, after the rats were maintained on the high-fat diet and retested with leptin on days 5 and 15, no effects of leptin on food intake were observed on either day (Fig. 3B and C) \( (\text{leptin treatment on day 5: } F_{1,9} = 0.04, P < 0.845; \text{day 15: } F_{1,9} = 1.52, P < 0.24557) \).

**DISCUSSION**

The major finding of this study is that the feeding response to peripheral leptin is affected by diet composition, being observed when rats are feeding on a low-fat diet, but not in rats consuming a high-fat diet.
When low fat-fed rats were tested with a high-fat diet, the hypophagic effect of leptin on the high-fat diet was present on day 1, but it disappeared by days 5 and 15. In contrast, when high fat-fed rats were tested with a low-fat diet, the feeding reduction was evident on days 1 and 15. These data suggest that ingestion of a high-fat diet abolishes the response to peripheral leptin and that dietary fat may induce “leptin resistance” within a short period of time. In addition, leptin decreased the hypothalamic NPY mRNA in low fat-fed rats but not in rats maintained on the high-fat diet.

The present study used a feeding regime that adapted rats to one diet and subsequently tested them on a diet that differed in its fat and carbohydrate composition. This approach allows the differentiation between a chronic signal related to adaptation to a diet from an acute pre- or postabsorptive signal related to the diet being consumed during the test period. This approach has been used in studies of enterostatin, CCK, and bombesin (13, 20, 27). Rats maintained on a high-fat diet exhibit reduced satiety responses to CCK and bombesin, regardless of whether they were tested with a high- or low-fat diet. Conversely, we showed that a chronic signal related to fat intake was necessary for the response to enterostatin (20). Our results here showed that the inhibitory response to the leptin was abolished in rats adapted to a high-fat diet, but it was restored immediately by giving a low-fat diet. Widdowson et al. (27) showed that leptin reduced feeding 1 wk after obese rats were switched from a high-fat diet to normal laboratory chow diet. Our current results suggest that acute ingestion of a first low-fat, high-carbohydrate meal is sufficient to restore the leptin response. In contrast, the leptin hypophagic effect in the present study was evident in rats adapted to the low-fat diet, but it disappeared within 5 days of changing to the high-fat diet. Because the response to leptin was still apparent when low-fat diet-adapted rats were naively tested on a high-fat diet, it suggests that a signal related to ingestion of a low-fat, high-carbohydrate diet is necessary for the response to leptin rather than there being an inhibitory response to dietary fat content of an immediate meal or the metabolic or endocrine response to the fat meal. That is, it suggests that there is an adaptive response to increasing the fat content of the diet that blocks the response to leptin and that this signal associated with carbohydrate feeding requires more than 24 h to disappear, but it will reappear very rapidly on introduction of a low-fat, high-carbohydrate diet.

As previously reported, dietary-induced obesity in mice and rats had increased circulating leptin, corticosterone, and insulin concentrations (1, 14, 27). Our data from rats killed 20 days after their diets were switched are consistent with this literature. Leptin significantly increased corticosterone and decreased hypothalamic NPY mRNA levels in rats fed the low-fat diet for 20 days after being switched from the high-fat diet. However, in the reverse situation, rats converted to the high-fat diet and maintained on this diet for 20 days did not show either a corticosterone or NPY mRNA response to leptin. Although leptin is known to affect multiple neuropeptide systems within the hypothalamus, including NPY, α-melanocyte-stimulating.
hormone, cocaine- and amphetamine-regulated transcript, and agouti-related peptide, the absence of any effect on NPY mRNA in rats adapted to a high-fat diet provides a mechanistic basis for the failure to observe the feeding response and provides evidence for “leptin resistance” within the hypothalamus. At this time, we do not know if the other target genes are similarly unaffected by leptin in the high-fat diet-adapted rat. However, if the leptin resistance is associated either with impairment in leptin transport into the central nervous system or lack of activation of the JAK-STAT signaling pathway, it would be expected that none of these target genes would show any response to leptin in the high-fat diet-adapted rat.

Our previous results showed that Osborne-Mendel and SS/Pl rats fed on a high-fat diet responded to leptin given intracerebroventriculally (21). Others have also shown normal or attenuated inhibitory responses to central injection of leptin in mice or rats fed a high-fat diet (26, 27). These data suggest that leptin resistance is more likely expressed at a peripheral level, an interpretation that is supported by direct comparison of responses to peripheral and central leptin in mice (26). The mechanism of peripheral leptin resistance remains to be determined. Banks et al. (2) have shown that leptin transport into the brain is lower in mice that become obese on a high-fat diet compared with mice that stay lean. The identity of the signal in high-fat-fed rats that impairs the response to leptin or the signal in low-fat, high carbohydrate-fed rats that permits the response to leptin is not known at the current time. It seems unlikely that it is related with any change of body fat mass because of the speed of the onset and disappearance of this leptin resistance. The current study implies that the diet per se, either directly or indirectly, changes the leptin sensitivity independent of the adipose weight. Recent studies in lean human subjects also suggest that dietary fat per se, independent of body mass index, may enhance leptin secretion (12). Because insulin secretion is likely to differ when ingesting the high-fat or low-fat diet, it is also possible that insulin itself might modulate the response to peripheral leptin.

A number of factors may contribute to the development of leptin resistance after introduction of a high-fat diet. These include the presence of a circulating antagonist or binding protein, alterations in clearance, transport into the brain, leptin-receptor downregulation, inhibition of JAK-STAT pathway, or activation of SOCS3 or other cytokine signal inhibitors (15). It seems possible that a high-fat diet enhanced clearance of leptin because the increment of circulating levels of leptin achieved after intraperitoneal injection of a high-fat diet was smaller in rats fed the high-fat diet compared with the low-fat diet. Leptin transport into the brain is saturable (3) and could be affected by dietary fat as suggested by the observations of Banks et al. (2). It is also possible that receptor downregulation or desensitization of the receptor affects the signaling pathway. We have recently shown that enhancement of leptin sensitivity after adrenalectomy results from both a constitutive activation of the JAK-STAT pathway and inhibition of the expression of the inhibitory SOCS3 gene (28).

Perspectives

High-fat diets have become a popular diet through which obesity may be induced in rodent models. As in other forms of obesity, it is associated with an increase in leptin secretion. It has been assumed that the apparent development of leptin resistance was related to the rapid deposition of excess body fat and the developing obesity. The current experiments suggest that the high-fat diet itself induces a state of resistance to peripheral leptin. Similar changes in responsiveness to other orexigenic and anorectic agents, e.g., CCK and enterostatin, have also been observed with changes in diet composition. With enterostatin, once again the changes in response to diet had a very rapid onset or disappearance. Such studies suggest that a signal associated with the ingestion of fat modulates the response to a number of peptides and metabolic signals that affects ingestive behavior. The identity of the “fat signal” is unclear. It could be either a direct response to a component of the diet; an olfactory, gustatory, sensory, or gastrointestinal neural response; or it could be endocrine in nature. The identification of this signal will provide significant insight into our understanding of body weight control.

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