Feeding and temperature responses to intravenous leptin infusion are differential predictors of obesity in rats

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Ruffin, Marie-Pierre, Tiziana Adage, Folkert Kuipers, Jan H. Strubbe, Anton J. W. Scheurink, and Gertjan van Dijk. Feeding and temperature responses to intravenous leptin infusion are differential predictors of obesity in rats. Am J Physiol Regul Integr Comp Physiol 286: R756–R763, 2004. First published December 4, 2003; 10.1152/ajpregu.00508.2002.—Obesity is frequently associated with leptin resistance. The present study investigated whether leptin resistance in rats is present before obesity develops, and thus could underlie obesity induced by 16 wk exposure to a liquid, palatable, high-energy diet (HED). Before HED exposure, male Wistar rats (weighing between 330 and 360 g) received intravenous infusions of 20 μg leptin 2 h before dark (~57 μg/kg rat). Relative to saline infusion, this caused a highly variable effect on food intake (ranging between −94 and +129%), with food intake suppression that appeared negatively correlated with HED-induced increases in body weight gain, caloric intake, adiposity, and plasma leptin levels. In contrast, leptin’s thermogenic response was positively correlated to body weight gain linked to weights of viscera, but not to adiposity. Before HED exposure, leptin unexpectedly increased food intake in some rats (fi+, n = 8), whereas others displayed the normal reduction in food intake (fi−, n = 7). HED-exposed fi+ rats had higher plasma leptin levels, retroperitoneal fat pad weight, HED intake, and body weight gain than fi− and chow-fed rats. These parameters were also higher in HED-exposed fi− rats relative to chow rats, except for plasma leptin concentrations. It is concluded that leptin’s reduced efficacy to suppress food intake could predict obesity on an HED. An unexpected orexigenic effect of leptin might potentially contribute to this as well.

leptin; obesity; thermogenesis; hypothalamus; diet

It is well established that most mammalian species are able to maintain their amount of body fat (i.e., a compartment that largely contributes to body weight) at a certain level over prolonged periods (11, 12, 35). Even profound modifications in adiposity, such as those associated with prolonged food restriction, overfeeding, or lipectomy, often lead to predictable behavioral and/or metabolic responses to restore the previous level of body fat. This phenomenon implies the existence of an adiposity-related feedback signal from fat stores to the brain (13). Evidence is accumulating that the adipose tissue factor leptin is a major candidate signal to inform the central nervous system (CNS) regarding the amount of body fat (5, 41). Leptin, a 167-amino-acid product of the OB gene (41), is produced mainly by adipocytes and secreted in the circulation in proportion to fat mass in many species, including rodents (8, 20) and humans (6, 7, 22). Leptin can reach the brain via a specific transport system (3, 31), and brain targets of leptin are, among others, located in the arcuate, ventromedial, and dorsomedial hypothalamic nuclei (32), i.e., all areas known to be involved in the control of energy homeostasis. Consistent with its presumed role of signaling the amount of fat to the CNS, administration of leptin decreases food intake and increases energy expenditure and thermogenesis, and thus promotes body weight loss in leptin-deficient (24) and genetically normal (28, 39) rodents. The fact that obese rodents and humans have high levels of circulating leptin (20, 22) could indicate a relation between the increase of fat mass and a malfunctioning of the leptin feedback loop to CNS regions involved in regulation of energy balance.

It is evident that the proportion of obese individuals in most developed countries has been on the rise throughout the last decade. The causes of the epidemic of obesity are as yet unknown, but factors like a change in lifestyle favoring a sedentary existence in combination with a palatable fat-rich high-energy diet (HED) have spurred the idea that human obesity is an example of a lifestyle-induced disease. Some individuals appear diet resistant, whereas others are thrifter in their capacity to extract/store energy from consumed food items and develop diet-induced obesity (DIO). In their study of the involvement of the leptinergic loop in the control of body weight regulation, Levin and Dunn-Meynell (15) found that the anorexigenic effect of leptin given in the lateral cerebral ventricle of rats was inversely related to the subsequent weight gain on an HED. These data therefore indicate that central leptin resistance can predispose obesity. In our study, we aimed at investigation of whether such a relation can be found when leptin is given peripherally. Therefore, we assessed the individual sensitivity to an acute intravenous infusion of leptin by measuring the well-known subsequent inhibition of feeding and increase in body temperature (24). In the present study, a subthreshold dose of leptin (20 μg, based on dose-response effects of leptin on food intake) was used, anticipating that some rats would react to the treatment, whereas others (presumably the DIO-prone ones) would not. The animals were offered, in addition to their normal laboratory chow, a palatable HED (based on a sucrose solution with emulsified corn oil) for 16 wk before investigating possible correlations between leptin sensitivity and body weight gain, energy intake, weights of several organs and fat pads, and plasma levels of several hormones/fuels indicative of the nutritional status.

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METHODS

Animals and surgery. Twenty-nine male Wistar rats from the breeding colony of the Department of Animal Physiology weighing 330-360 g at the beginning of the study were individually housed in Plexiglas cages (25 × 25 × 30 cm) with ad libitum access to standard laboratory chow (Hope Farms) and water. The rats were maintained under a 12:12-h light-dark cycle (lights on from 0500 to 1700) in a temperature-regulated room (20 ± 2°C) and were handled and weighed each day. After 1 wk of habituation to housing conditions, all rats received a permanent heart catheter (under isoflurane anesthesia) inserted in the right jugular vein, as previously described (36). This technique allows blood sampling and intravenous infusions in undisturbed freely behaving rats (36). All experiments were started after the rats surpassed their preoperative weights (−2 wk). During this time, they were habituated to experimental procedures (e.g., blood sampling/infusion and insertion of a rectal probe for assessment of body temperature). These, and all other methodologies, are in accordance with the guidelines of the Ethical Committee of the University of Groningen, as well as with the “Guiding principles for research involving animals and human beings” (1a).

Dose-response effects of leptin. In a counterbalanced design, with at least 4 days between successive experiments, six rats were intravenously infused before the dark phase with saline (0.25 ml) or saline (0.25 ml) containing either 20 μg (~57 μg/kg rat), 50 μg (~143 μg/kg rat), or 100 μg (~350 μg/kg rat) of leptin (human leptin, PeProTech: lot no. 116901). Specifically, on a test day, food hoppers were removed from cages 2 h before lights off. Immediately thereafter, animals were placed briefly on the lap of the investigator during which intravenous infusion of leptin or vehicle took place over a 30-s interval. At lights off, food hoppers were returned to the cages, and hopper weights were assessed at 2, 4, and 12 h of the dark phase.

Leptin sensitivity testing. From the remaining group of 23 rats, a blood sample of 0.5 ml was taken via the jugular vein cannula in the middle of the light phase for determination of the plasma leptin concentration. Thereafter, rats were semirandomly assigned to two groups such that the mean body weights of groups were identical. In one group of rats (n = 15), the individual sensitivities for 20 μg of intravenously infused leptin were assessed to affect 2-h food intake in the dark phase. The dose of leptin (20 μg) and time window over which food intake was assessed (2 h) were based on the outcome of the dose-response study (see RESULTS). Leptin effects on food intake were compared with the effects on food intake obtained with saline. Leptin and saline infusions were performed during 2 days that were separated by 1 wk, and the order of infusions was semirandom such that about half of the rats was treated with leptin on the first test day, whereas the others were treated with leptin on the second test day. Sensitivity to saline was assessed on alternate days. On a test day, infusion procedures and food hopper registration at 2 h in the dark phase were similar as in the dose-response study. In addition, 1 h after intravenous infusion, rats were shortly placed in an empty tub during which they had a lubricated temperature probe gently inserted rectally (4 cm, for 2 min) for assessment of body temperature. Between experimental days, 2-h food intake of rats not preceded by any manipulation was assessed to study baseline food intake of rats.

HED exposure. After completing the individual leptin sensitivity tests, these animals received a liquid, highly palatable HED rich in dietary fat, in addition to their normal laboratory chow, over a period of 16 wk. Instead of receiving the HED, the remaining rats (n = 8) continued feeding on the standard laboratory chow and served as controls for the HED group. The HED has been adapted from Smith and colleagues (34), who, using a fat emulsion in water blended with a mixture of saccharine/glucose, were able to render rats hyperphagic and obese. The diet used in the present study consisted of a 10% sucrose solution in water mixed with a gradually increasing proportion of corn oil, from 4% volume (10 days) to 8% volume (2 wk), 16% volume (2 wk), and finally 32% volume (until the end of the study at 16 wk). Soy lecithin and Tween 80 were added for effective emulsion of corn oil in the sucrose solution. No extra protein was added to the oil emulsion because rats on the HED maintained the ability to select their normal chow as well. From the data of Smith and colleagues and our own pilot data, it appeared that rats, while ingesting the oil emulsion, never suppressed their chow intake below 50% of control values. Because the chow used in the present study contained 21.4% proteins as total energy, they still ate more protein (5.5%) than the minimum requirement (i.e., 5%) for maintenance (23). Table 1 describes the detailed composition of the diets and their macronutrient proportions and energy contents. Water was available ad libitum for both groups.

The individual leptin sensitivity test was performed with infusions performed at 2 h before the dark phase (i.e., a semistarved condition), which is a time when the circulating level of leptin is normally low relative to the period just after the dark phase (i.e., a postprandial condition; see Ref. 1). Because the elevated circulating leptin level as a result of infusion might cue to the postprandial condition, it would be of interest to correlate the individual leptin sensitivity before HED exposure with several postprandial parameters at the end of the HED exposure period. For this reason, chow and HED rats were killed at the end of the 16-wk HED exposure ~2 h after lights on. After decapitation, blood was collected for determination of plasma levels of free fatty acids, triglycerides, cholesterol, glucose, leptin, insulin, and corticosterone. Carcasses were dissected for assessment of weights of livers, gastrointestinal tracts, and of retroperitoneal and epididymal fat pads.

Table 1. Composition of the diets

<table>
<thead>
<tr>
<th></th>
<th>HED</th>
<th>Chow</th>
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<tbody>
<tr>
<td></td>
<td>4% Fat</td>
<td>8% Fat</td>
</tr>
<tr>
<td>Water, ml</td>
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<td>920</td>
</tr>
<tr>
<td>Sucrose, g</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Corn oil, ml</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Lecithin-98, g</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Tween 80, ml</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Percentage of macronutrients (expressed as mass and energy) and total energy content of the liquid HED and of standard laboratory chow

| Carbohydrate (mass-energy), % | 8.7–50.2 | 8.4–33.4 | 7.8–20.1 | 6.8–11.3 | 58.5–62.3 |
| Protein (mass-energy), % | 0 | 0 | 0 | 0 | 21.4–22.8 |
| Fat (mass-energy), % | 3.8–49.8 | 7.4–66.6 | 13.7–79.9 | 23.6–88.7 | 6.2–14.9 |
| Energy content, kJ/g | 2.66 | 3.72 | 5.59 | 8.69 | 17.8 |

*Adapted from Smith et al. (34). Lecitin was from de Natuurwinkel. Tween 80 was from Merck. HED, high-energy diet.
Biochemical determinations of plasma hormones and fuels. Collected blood was immediately placed in ice-cooled borosilicate tubes containing 8% (of blood volume) of a solution of aprotonin (10,000 IU/ml)-EDTA (0.5 g/ml). Plasma samples (after centrifugation for 15 min at 1,500 g and 4°C) were stored at −80°C. Plasma levels of insulin and leptin were measured by commercial RIA kits (RI-13K and RL-83K, respectively, Linco Research); plasma concentrations of glucose, triglycerides, free fatty acids, and total cholesterol were measured using commercial kits (Boehringer Mannheim, Mannheim, Germany); plasma corticosterone was measured using HPLC with an ultraviolet detector.

Data analyses. The dose-response effects of leptin on cumulative food intake were analyzed with repeated-measures ANOVA using a within-subject design (i.e., all doses were tested in each animal). Post hoc analyses were performed by the least significant difference (LSD) test at individual time points. Effects of diet on body weight gain were analyzed with repeated-measures ANOVA, followed post hoc by the LSD test at individual time points. Final energy intake, body weight gain, and end-point metabolic and hormonal parameters were analyzed with one-way ANOVAs with post-hoc LSD tests. All data are expressed as means ± SE, and effects are considered significant at P < 0.05.

Leptin sensitivity for altering body temperature was calculated as the within-subject’s difference in rectal temperature after leptin and saline infusions (leptin temperature – saline temperature). Leptin sensitivity for altering food intake was calculated as the difference in food intake after leptin and saline infusion, relative to effects after saline infusion: [(food intake after leptin injection – food intake after saline injection)/food intake after saline injection] × 100. Correlations between leptin sensitivity and other parameters were assessed by means of a two-tailed Spearman’s rank correlation test.

RESULTS

Dose-response effect of leptin. Repeated-measures ANOVA revealed dose [F(3,15) = 4.505, P = 0.019] and time [F(2,10) = 684, P < 0.0001] effects of intravenous leptin infusion on cumulative food intake over 2, 4, and 12 h in the dark phase. No interactions between dose and time were found, presumably because the effect of leptin on food intake became visible after 2 h in the dark phase and did not change dramatically thereafter (Fig. 1). Post hoc analyses revealed that, relative to saline infusion, leptin dose at 50 and 100 µg significantly reduced cumulative food intake at 2 and 4 h, whereas only the highest dose of leptin also reduced food intake over 12 h. After infusion of 20 µg leptin, however, some animals clearly responded with reduced food intake, whereas others did not (and, in fact, increased food intake relative to saline). Thus, based on the observations that leptin altered food intake within the first 2 h without major compensation later on and that a highly variable effect was achieved with the lowest dose, we decided to assess the effects of 20 µg leptin (relative to saline) on 2-h food intake and body temperature as dependent variables for individual leptin sensitivity.

Leptin sensitivity test. The feeding response over the first 2 h of the dark phase after intravenously infused leptin (20 µg) was not different from that after intravenous saline infusion (3.5 ± 0.4 and 4.3 ± 0.5 g, respectively). Large differences were observed in the changes in food intake after leptin infusion relative to saline infusion (ranging from −94% to +129%), with seven rats reducing their food intake (fi−) and as much as eight rats increasing their food intake (fi+) to leptin relative to saline treatment. Although variation in the absolute feeding response to leptin undoubtedly contributed to these effects (i.e., fi+ rats ate 4.9 ± 0.8 g and fi− rats ate 3.0 ± 0.5 g after leptin), there was, however, also a large variation in the absolute feeding response after saline infusion (i.e., fi+ rats ate 3.3 ± 0.4 g and fi− rats ate 5.5 ± 1.2 g after saline infusion). This means that a relatively low level of food intake after saline infusion contributed to the orexigenic effects of leptin as well. These relatively low levels of food intake in some animals are probably innate since they were also found in these animals when no infusion was given. In fact, comparison between 2-h food intake after saline infusion and no infusion yielded a small overall difference that was significantly smaller (P = 0.025) than the overall difference between 2-h food intake after leptin and no infusion. These data therefore seem to indicate that the variable food intake responses among rats are reproducible and not caused by differential responses to experimental procedures per se.

The temperature response to leptin infusion (37.10 ± 0.11°C) was not different from that after saline infusion (36.76 ± 0.11°C) either. The individual variability of temperature differences after leptin and saline injection ranged from −0.3°C to +0.8°C, with only 4 rats reducing their rectal temperature to leptin, and 11 rats increasing their rectal temperature in relation to saline treatment.

Effects of diet on obesity-related parameters. Before exposure to the HED, the mean body weight of rats was 471.9 ± 6.0 and 461.4 ± 8.5 g in the pre-HED and chow group, respectively. Baseline plasma leptin levels were 6.3 ± 0.9 and 5.9 ± 0.5 ng/ml in the pre-HED and chow group, respectively. Significantly higher body weight gain over time in the HED group relative to the chow group [diet × time: F(4,84) = 14.78, P < 0.0001] appeared after 8 wk and progressively increased during the ensuing 8 wk of diet exposure (Fig. 2A). At the end of the experiment, mean body weight of HED rats was 602.2 ± 11.4 vs. 532.6 ± 10.3 g in chow rats (P < 0.01). This corresponded to a 82% higher (P < 0.01) body weight gain of HED rats (130.2 ± 7.8 g) relative to the chow rats (71.2 ± 9.9 g). The initial daily energy intake on standard chow was 425.1 ± 16.8 kJ. During the 16 wk of diet exposure, the daily energy intake of HED rats greatly increased to reach 814.0 ± 30.1 kJ/day (91% increase relative to chow rats; Fig. 2B, P < 0.001) during the last week of diet exposure. During this final week of HED exposure, 25.8% of the energy intake originated from standard chow and the remaining 74.2% from the HED. In HED-exposed rats relative to chow rats, plasma glucose (116.1 ± 1.8 and 106.8 ± 4.0 mg/dl, respectively, P <
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Fig. 2. A: body weight change in laboratory chow-feeding rats (○) and rats that had access to a liquid palatable high-energy diet (HED) adapted from Smith et al. (34), which was available in addition to their standard laboratory chow (▲). *P < 0.05 and **P < 0.01 vs. chow group. B: mean daily energy intake (kJ/day) of HED-exposed rats during the last week and of chow rats. Hatched bars, the amount of HED-exposed rats during the last week and of chow rats. Hatched bars, the contribution of HED to total food intake. Open bars, the amount of (kJ/day) of HED-exposed rats during the last week and of chow rats. Hatched

al. (34), which was available in addition to their standard laboratory chow (that had access to a liquid palatable high-energy diet (HED) adapted from Smith et

0.05) and leptin (19.7 ± 0.7 and 9.5 ± 1.3 ng/ml, respectively, P < 0.001) levels and weights of retroperitoneal (13.8 ± 1.1 and 6.6 ± 0.5 g, respectively, P < 0.001) and epididymal (16.1 ± 0.9 and 11.3 ± 0.6 g, respectively, P < 0.01) fat pads and the gastrointestinal tract (36.8 ± 1.3 and 32.2 ± 1.0 g, respectively, P < 0.05) were significantly increased. Plasma levels of free fatty acids, triglycerides, cholesterol, corticosterone, and insulin, as well as liver weights at the end of the diet exposure, were not significantly different between HED and chow rats.

Overall correlations in HED rats. The efficacy of leptin to alter body temperature relative to saline was positively correlated with its efficacy to alter food intake (Spearman’s r = 0.515, P < 0.05, Fig. 3). Thus the higher the increase in rectal temperature resulting from leptin infusion, the weaker the effect of leptin was to suppress food intake relative to intravenous saline infusion. No significant correlations were found of the efficacy of leptin to alter food intake or temperature with basal plasma leptin levels, body weight gain, or energy intake before leptin sensitivity tests and HED exposure, nor were the pre-HED plasma leptin levels correlated to any parameter analyzed after the HED period (data not shown).

The percentage of change in food intake in response to leptin relative to saline correlated positively with body weight gain over the period of HED exposure (Spearman’s r = 0.637, P < 0.05, Fig. 5A), indicating that the more the animals responded to the leptin infusion with increases in body temperature, the more they also became overweight because of HED exposure. There were no correlations of the temperature response to leptin infusion in relation to saline with any parameter linked to adiposity. Instead, analyses of leptin effects on body temperature were found to be highly correlated with liver weight (Spearman’s r = 0.688, P < 0.01, Fig. 5B) and weight of the gastrointestinal tract (Spearman’s r = 0.700, P < 0.01, Fig. 5C).

Grouping of leptin responders according to food intake. Because the rats responding to leptin (relative to saline) with a reduction in food intake (fi−, n = 7) and an increase (fi+, n = 8) in food intake were evenly distributed, their end-point metabolic, hormonal, and ingestive parameters after HED exposure were grouped accordingly and compared with those in chow rats. Thus, at the end of HED exposure, fi+ rats had significantly higher plasma levels of leptin, weights of epididymal and retroperitoneal fat pads and gastrointestinal tracts, daily energy intake, and body weight gain over the course of HED exposure than fi− and chow rats. Relative to chow rats, fi+ rats had higher retroperitoneal fat pad weight, daily energy intake, and body weight gain over the course of HED exposure, whereas plasma leptin levels were not different (Table 2). In spite of the significant differences between fi+ and fi− rats after HED exposure, plasma leptin levels of fi+ and fi− rats before HED exposure were identical, i.e., 6.3 ± 0.9 and 6.3 ± 0.5 ng/ml, respectively.

DISCUSSION

Sixteen weeks exposure of adult male Wistar rats to a liquid, highly palatable HED rich in dietary fat consistently increased body weight gain (by 81%), energy intake (by 92%), plasma leptin concentrations (by 109%), retroperitoneal fat mass (by 108%), and weight of the gastrointestinal tract (by 14%) relative to control rats that remained feeding standard laboratory chow. Plasma leptin levels in the HED group were highly correlated to both body weight and retroperitoneal fat mass (data not shown), similarly to what has already been reported in other rodent DIO models (8, 15–18). Relative to chow rats, plasma glucose significantly increased (by 8%) in the HED rats

Fig. 3. Effect of iv leptin (20 μg) infusion relative to saline infusion on rectal temperature (ΔTemp (°C): body temperature after leptin infusion – body temperature after saline infusion) and food intake (%Δ 2h-FI: [(2-h food intake after leptin infusion – 2-h food intake after saline infusion)/(2-h food intake after saline infusion) × 100]). Spearman’s r = 0.515, P < 0.05.
without a concomitant increase in plasma insulin (although the latter is curvilinear related to body weight gain and adiposity). This suggests that rats on the HED developed slight glucose intolerance, a phenomenon frequently observed in obese subjects (26). Also often associated with obesity and increased dietary fat intake are increases in circulating fat fuels. However, the HED failed to increase plasma levels of free fatty acids, cholesterol, and triglycerides in the present study, and this may probably be because of the use of the relatively “healthy” corn oil as the fat basis (e.g., Ref. 14) and/or the choice of animal species used in the present study. Thus it seems that this model mimics a relatively mild form of obesity, i.e., increased fat deposition and disturbed glycemic regulation without dislipidemia.

Numerous reports suggest that leptin produces anorexia and stimulates thermogenesis, both responses aimed at promoting weight loss (e.g., Ref. 38). A leading hypothesis regarding obesity proposes that these leptin responses are blunted in obesity-prone subjects. In the aim of investigating a possible relationship between the efficacy of the leptin feedback loop and the development of obesity, rats were intravenously infused with 20 μg leptin, a subthreshold dose for reducing food intake relative to saline treatment. Use of such a dose would identify rats (with food intake suppression and an increase in body temperature as read-out parameters) that are very sensitive to leptin and those that are not. Although the intravenously infused leptin did not cause significant changes in food intake or rectal temperature across all animals relative to the saline condition, there were large individual differences in the magnitude of these responses. Consistent with the above-stated hypothesis was the observation in the present study that a relatively weak suppression of food intake by leptin infusion correlated with increased HED intake, body weight gain on the HED, and parameters linked to increased adiposity, such as retroperitoneal fat mass and the plasma leptin concentration at the end of HED exposure. The finding that hyperphagia persisted in the rats that developed hyperleptinemia strengthens the hypothesis that obesity is associated with leptin’s inability

![Fig. 4](http://ajpregu.physiology.org/). The effect of iv leptin infusion (20 μg) relative to saline infusion on food intake [%Δ 2h-Fi: [(2-h food intake after leptin infusion – 2-h food intake after saline infusion)/2-h food intake after saline infusion] × 100] before exposing rats to the HED plotted against several indexes (vertical axis) of obesity at the end of HED exposure (assessed 2 h after lights on). A: body weight gain; Spearman’s r = 0.532, P < 0.05. B: daily energy intake; Spearman’s r = 0.779, P < 0.001. C: plasma leptin concentration; Spearman’s r = 0.593, P < 0.05. D: retroperitoneal fat mass; Spearman’s r = 0.693, P < 0.01.

![Fig. 5](http://ajpregu.physiology.org/). Effect of iv leptin (20 μg) infusion relative to saline infusion on rectal temperature [Δ Temp (°C): (body temperature after leptin infusion – body temperature after saline infusion)] before exposing rats to the HED plotted against several indexes (vertical axis) of obesity at the end of HED exposure (assessed 2 h after lights on). A: body weight gain; Spearman’s r = 0.637, P < 0.05. B: liver weight; Spearman’s r = 0.688, P < 0.01. C: gastrointestinal (GI) tract weight; Spearman’s r = 0.700, P < 0.01.
to suppress feeding. Our data are consistent with those of Levin and Dunn-Meynell (15), who demonstrated reduced efficacy of centrally administered leptin to reduce food intake in DIO rats relative to diet-resistant ones. One important implication of their study is that rats are DIO-prone because of reduced relative to diet-resistant ones. One important implication of their study is that rats are DIO-prone because of reduced relative to diet-resistant ones.

An intriguing outcome of our study, as opposed to the study of Levin and Dunn-Meynell (15), was the capacity of intravenously infused leptin to increase 2-h food intake relative to saline treatment in at least 50% of the rats tested. We are aware of the fact that an orexigenic (fi+) effect of leptin is at odds with the mainstream lipestat idea of leptin acting as a negative feedback signal in the CNS to stimulate weight loss (5, 13, 38), and these data as such might be meaningless. However, a counterargument may be that doses of leptin capable of causing potent reductions in food intake are not physiological and mask potential orexigenic effects of leptin. As judged from the 31% higher body weight gain and 51% heavier retroperitoneal fat pads (i.e., an indicator of total adiposity) in the fi+ rats relative to the fi− rats, the fi+ animals became clearly more obese on the HED than the rats that responded with a relative reduction in food intake to leptin treatment (fi−). Because the fi+ rats also had 67% higher plasma leptin levels than the fi− rats at the end of HED exposure, this opens the important possibility that a potential orexigenic effect of leptin contributed to the 21% higher energy intake and the concomitant development of obesity. At this point, it needs to be mentioned that fi+ rats had a relatively low level of 2-h food intake after saline intake and that this low level of food intake somewhat contributed to the fi− effect of leptin. Because this low level of food intake after saline was also observed when no infusion was given, this could be a trait characteristic of the DIO-prone rats in our study. In future experiments, dose-response studies of leptin with food intake and body temperature as dependent variables have to be performed at the end of HED exposure to fully substantiate the role of leptin in proneness to attract DIO.

Whereas reduced efficacy of leptin to suppress food intake may result from a blunted ability of leptin to downregulate arcuate hypothalamic neuropeptide Y mRNA expression (15), the mechanism underlying an fi+ effect is not known to date. An fi+ action of leptin may be consistent with the data of Shizgal and colleagues (10, 33), who found that electrical stimulation of lateral hypothalamic neural networks in rats increases sucrose ingestion, which can either be attenuated or augmented by intracerebroventricular leptin administration, depending on the location of the electrical probe. Thus, depending on the activity of neuronal networks in the lateral hypothalamus, leptin may either have orexigenic or anorexigenic effects. The lateral hypothalamus contains, among others, neuronal cell bodies that express melanin-concentrating hormone and orexin (27, 30), i.e., neuropeptides that have profound orexigenic and arousing effects. It remains to be investigated whether there are certain conditions under which the synthesis of these orexigenic neuropeptides can be upregulated by leptin, rather than suppressed (29), providing some of the underpinnings of the observed phenomena in the present study.

Orexigenic effects of leptin have also been observed by Ammar et al. (2), who demonstrated that intracerebroventricular lepasine administration in rats trained to ingest a sucrose solution intraorally is able to increase this intraoral uptake of sucrose. When this sucrose solution is provided by a bottle, however, the same dose of leptin reduced sucrose ingestion (2). Based on these and other observations, one could propose that leptin reduces the appetitive or “wanting” component of ingestion (i.e., underlying reduced sucrose uptake from a bottle) while at the same time increasing the consummatory or “liking” component (i.e., when sucrose was provided intraorally, and thus bypassing the appetitive phase). These components of ingestive behavior have previously been introduced by Berthoud and Robinson (4). Provided that both phases of ingestion determine the total amount of food intake, this idea gives a new perspective to the concept of “leptin resistance” leading to the development of hyperphagia and DIO. Thus we speculate that

### Table 2. Metabolic, hormonal, and ingestive parameters linked to the nutritional status in rats fed chow or an HED diet

<table>
<thead>
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<th>Parameter</th>
<th>Chow</th>
<th>fi−</th>
<th>fi+</th>
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<td>FFA, mmol/l</td>
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<td>Corticosterone, μg/dl</td>
<td>10.1±1.3</td>
<td>8.1±1.7</td>
<td>7.5±1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.69±0.23</td>
<td>2.94±0.53</td>
<td>4.00±0.56</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>106.8±2.8</td>
<td>115.6±3.2</td>
<td>118.4±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>9.5±0.9</td>
<td>14.5±1.8</td>
<td>24.3±3.3*†</td>
<td>10.4, P = 0.001</td>
</tr>
<tr>
<td>Gastrointestinal tract, g</td>
<td>32.2±1.0</td>
<td>33.7±1.1</td>
<td>39.9±1.9*†</td>
<td>6.8, P &lt; 0.006</td>
</tr>
<tr>
<td>Liver, g</td>
<td>16.2±0.7</td>
<td>16.4±0.7</td>
<td>17.9±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Epididymal fat, g</td>
<td>11.3±0.6</td>
<td>14.5±1.2</td>
<td>17.5±1.4†</td>
<td>7.3, P = 0.004</td>
</tr>
<tr>
<td>Retroperitoneal fat, g</td>
<td>6.6±0.5</td>
<td>11.1±0.8†</td>
<td>16.8±1.7*†</td>
<td>18.9, P &lt; 0.0001</td>
</tr>
<tr>
<td>Body weight gain during diet exposure, g</td>
<td>71.2±10.6</td>
<td>111.5±10.6†</td>
<td>146.6±10.5*†</td>
<td>13.2, P &lt; 0.0001</td>
</tr>
<tr>
<td>Daily energy intake during final week, kJ</td>
<td>352.0±13.9</td>
<td>752.5±28.5†</td>
<td>912.8±33.1†</td>
<td>125.0, P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acid. Nutritional status was assessed in rats fed normal laboratory chow (n = 8) and rats exposed to an HED that responded either with reduced food intake (fi−, n = 7) or increased food intake (fi+, n = 8) to iv leptin infusion (20 μg/0.25 ml saline) in relation to iv saline infusion. Significant differences were assessed by one-way ANOVA, followed post hoc by the least significant difference test. Differences between fi− and fi+ rats. †Significant differences between HED (fi− or fi+) and chow rats.
fi+ rats in our study became diet-induced obese because they were more “consummatory” instead of “appetitively” driven. Interestingly, rats rendered diet-induced obese in another study showed markedly reduced food anticipatory activity (25), which may be consistent with low appetitive drive as well. The aforementioned finding that the fi+ rats in the present study displayed a relatively low level of food intake after saline infusion might be a consequence of low “appetitive drive” as well.

With respect to body temperature as a dependent variable for leptin sensitivity, a completely different picture emerged. Opposite to what we expected, we observed that the high efficacy of leptin to suppress food intake coincided with a relatively weak effect of leptin to increase body temperature. At the moment, we have no idea of the mechanisms in which the opposing sensitivities of leptin to increase body temperature or to reduce food intake are related, but probably find their causation in activation of discrete neuronal systems by leptin, which can influence food intake and body temperature independently of one another (see Ref. 19). One implication, however, could be that differences in leptin-induced thermogenesis do not account for the differences in the satiating properties of leptin (38), although the temporal pattern of the temperature responses needs to be investigated more closely (e.g., also during food intake) to fully substantiate this argument. Besides these effects, we observed that leptin’s efficacy to increase body temperature was positively correlated to body weight gain on the HED. Further correlation analyses revealed that the weight of visceral organs (including the liver and gastrointestinal tract), but not the weight of adipose tissue mass, contributed to this effect. We speculate that the correlation between leptin’s thermogenic action and weight gain reflects a metabolic response aimed at counterregulating weight gain seemingly unrelated to adiposity, even before these animals are actually challenged with the HED. The idea that the liver (9) and the gastrointestinal tract (37) are sensitive signaling organs able to detect subtle changes in nutritional status intuitively fits this possibility.

In summary, our study demonstrates that the susceptibility to develop obesity in rats fed a palatable HED is inversely correlated to the leptin efficacy by which it is able to reduce food intake, but not to increase rectal temperature. Because it was previously demonstrated that the categorization of rats between DIO-prone and diet-resistant ones depends on a different genetic background (16–18), leptin sensitivity and perhaps the orexigenic effects of leptin are probably behavioral/physiological manifestations of it and therefore participate in the resulting existence of regulated body weight (15). Future studies may be directed at pin-pointing the CNS control mechanisms that either prevent or propagate the development of obesity. One implication of the present study could be that caution needs be exercised in the aim of treating obesity with drugs that tie into the leptin signaling cascade. As a matter of fact, the data presented in this study might directly provide a mechanism for the failure of leptin as a therapeutic agent in obesity-prone humans (21).

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