An acute method to test leptin responsiveness in rats

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Desai BN, Harris RB. An acute method to test leptin responsiveness in rats. Am J Physiol Regul Integr Comp Physiol 306: R852–R860, 2014. First published March 26, 2014; doi:10.1152/ajpregu.00548.2013.—Continuous subcutaneous administration of leptin normalizes blood glucose levels in rodent models of Type 1 and Type 2 diabetes independent of changes in food intake, body weight, and plasma insulin. We tested whether an acute intravenous leptin infusion changed blood glucose in normal and diet-induced leptin-resistant rats to determine whether this measure could be used as a marker of leptin sensitivity. Leptin-responsive chow-fed rats and diet-induced leptin-resistant male Sprague-Dawley rats were fitted with thoracic jugular vein catheters. Four days after surgery, conscious rats were infused intravenously with either saline for 32 min, low-dose (LD) leptin (1.9 μg·kg⁻¹·min⁻¹) followed by high-dose (HD) leptin (3.8 μg·kg⁻¹·min⁻¹) for 16 min each, or only HD leptin for 16 min. There was no change in blood glucose after an acute intravenous infusion of either LD leptin or HD leptin alone for 16 min. An intravenous infusion of LD followed by HD leptin for 16 min each significantly decreased serum glucose in leptin-responsive rats but not in leptin-resistant rats. Leptin infusions increased serum leptin in all rat groups but had no effect on plasma glucagon or 12-h weight gain and energy intake in any group of rats. These results show that leptin has an acute glucose-lowering effect that reflects the leptin responsiveness of the rat. This effect is consistent across controls and different leptin-resistant rat models, and the acute nonlethal test provides a novel method of testing leptin responsiveness in rats.

blood glucose; leptin resistance; leptin infusion

THE ADIPOKINE LEPTIN, discovered in 1994 (37), has been identified as a critical physiological regulator of energy balance (12). Plasma leptin circulates at concentrations proportional to adipose mass and has been shown to act centrally to inhibit food intake and increase energy expenditure (21). Despite physiological systems in place to regulate energy homeostasis, approximately one-third of adults in the United States are classified as obese (20). Leptin deficiency is rare and is associated with severe early childhood obesity (22). Most obese adult individuals have high circulating concentrations of endogenous leptin; however, the hormone is unable to prevent an increase in fat mass (4), thus rendering these individuals “leptin resistant” (4).

Rodents and other animal models have been extensively used to understand the physiological basis of leptin’s role in body weight regulation and of the obese leptin-resistant phenotype (7, 11, 15, 33). Most studies of this kind involve the measurement of leptin sensitivity as a marker differentiating between a leptin-responsive and a leptin-resistant state. There are a number of methods used presently to measure leptin sensitivity in rodents. These include, but are not limited to, the measurement of food intake over 12–48 h after a leptin injection (10); monitoring 24-h energy expenditure and respiratory exchange ratio (R.E.R) (3); measurement of body weight, carcass fat, lean tissue, or liver glycogen after 3–4 wk of leptin treatment (11, 31); and finally, the measurement of phosphorylation of hypothalamic signal transducer and activator of transcription 3 (STAT3) 15–30 min after leptin injection (21). The activation of STAT3, a transcription factor, is considered an accepted marker of leptin receptor (ObRb) activation (24). These current methods, however, have a number of common technical disadvantages. First, most in vivo methods require chronic observations for up to 48 h before analysis. Second, some involve euthanasia of the experimental animals, making them unavailable to test for other measures in the same experiment.

The main objective of the studies described here was to identify an acute nonlethal method to test leptin responsiveness in rats. There is evidence that leptin acts both centrally and peripherally to normalize blood glucose. Pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus are glucose-sensing neurons (25), and leptin has been shown to act directly and indirectly on POMC neurons to normalize blood glucose and glucagon levels in mice (2). Similarly, chronic peripheral subcutaneous or central administration of leptin normalizes circulating blood glucose in rodent models of Type 1 and Type 2 diabetes in 7–12 days. This effect is independent of changes in body weight, food intake, or circulating concentrations of insulin (5, 36). These studies taken together provide strong evidence for glycemic stability with chronic leptin administration in diabetic rat models. In this paper, we extend these data to investigate whether acute, low-dose peripheral infusions of leptin lower blood glucose in normal leptin-responsive rats. The experiments were designed to test this acute leptin effect on blood glucose as a marker of leptin responsiveness.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 270–300 g (Harlan Laboratories, Indianapolis, IN) were housed individually in hanging wire mesh cages with lights on for 12 h each day from 07:00 h in a room maintained at 23°C. The animals had free access to chow (3.3 kcal/g, Harlan Teklad Rodent Diet 8604) and water throughout the study unless stated otherwise. Each rat had a Nylabone (Nylabone Products, Neptune, NJ) in their cage for enrichment. All animal procedures were approved by the Institutional Animal Care Use Committee of Georgia Regents University. Body weights were measured daily for each experiment.

Experiment 1. This experiment was designed to test the effect of acute low- and high-dose venous leptin infusion (recombinant rat leptin; R&D Systems, Minneapolis, MN) on serum glucose to determine whether circulating leptin levels had an acute effect on blood glucose concentrations. In pilot studies, increasing doses of leptin were tested for their effect on blood glucose. Doses of 1.5, 5.5, and 7.5 μg·kg⁻¹·min⁻¹ had no effect on blood glucose when each infusion was given independently. When an infusion of 1.9 μg·kg⁻¹·min⁻¹ was followed by an infusion of 3.8 μg·kg⁻¹·min⁻¹, there was a...
significant drop in blood glucose concentration and therefore a combination of the two doses were used in subsequent experiments.

A total of 24 male Sprague-Dawley rats (2 cohorts of 12 rats each) were housed and maintained as described above. The rats were anesthetized with isoflurane and given a subcutaneous injection of 2 mg/kg ketoprofen (Ketofen, Fort Dodge Animal Health, Fort Dodge, IA) as an anesthetic before the surgery. Each rat was fitted with a thoracic jugular vein catheter (Braintree Scientific, Braintree, MA). Once fitted, catheter placement was tested by drawing a small volume of blood into a 1-ml blunt needle syringe, and the catheter was filled with heparinized saline-glycerol (1:1) (250 U/ml heparin) before closing the open end with a solid catheter block.

Four days after surgery, four rats at a time were moved to another room and housed individually in shoebox cages for the infusion. The rats had free access to food and water until they were moved to the testing room. The average body weight of the rats at the time of infusion was 298 ± 5 g. The catheters were attached to infusion pumps (NE-1000 Programmable Single Syringe Pump; New Era Pump Systems, Farmingdale, NY) at 12:00 pm. The rats’ tails were then clipped and blood glucose was measured every 15 min using a glucometer (Easy-Gluco Glucose Meter-2657A; US Diagnostics, New York, NY) until a stable baseline reading was established. After three additional baseline readings 5 min apart, 0.9% saline or low-dose leptin infusion was started (LD: 1.9 μg·kg⁻¹·min⁻¹ or 0.8 μl·100 g⁻¹·min⁻¹) and blood glucose was measured every 2 min. After 16 min, the rate of infusion was doubled (HD: 3.8 μg·kg⁻¹·min⁻¹ or 1.9 μl·100 g⁻¹·min⁻¹) and continued for another 16 min, with blood glucose measured every 2 min. Total infusion volumes of leptin delivered (low dose: 48–56 μl; high dose: 96–112 μl) were extremely small compared with the total blood volume of 27–30 ml. The rate of saline infusion matched the rate of leptin infusion. At the end of the total 32-min infusion, a 0.5-ml blood sample of tail blood was collected to measure serum glucose, leptin, and insulin. Serum leptin and insulin were measured by radioimmunoasaysay (rat leptin RIA kit and rat insulin RIA kit; Millipore, Billerica, MA). An infusion time of 32 min was selected based on the outcome of the pilot experiment.

Each rat was infused twice, once with saline and once with leptin. Half of the rats received leptin and the other half received saline on the first day of infusion, and the treatments were switched on the second day so that each rat acted as its own control. The two infusions were separated by 2 days to allow stabilization of body weights and any other metabolic parameters that may be affected by the acute infusion. On the first day of infusion, a group of four rats were divided into two treatment groups: half of the rats were infused for 32 min with saline while the other half were infused with leptin (infusion day I). After 2 days, the treatments were switched (infusion day II). A total of four rats were taken out of the study because they chewed up their catheters.

Experiment 2. Diet-induced obese mice and rats have been shown to develop peripheral leptin resistance (33). This study was designed to test the effect of LD and HD venous leptin infusion on serum glucose in leptin-responsive rats as well as leptin-resistant rats, and to determine whether this measure could be used as an acute marker to differentiate between the two states of leptin sensitivity.

Leptin resistance was induced by dietary means. We have previously reported that rats given a choice of chow, lard (9 kcal/g), and 30% sucrose solution (1.2 kcal/ml) (Choice Diet) become leptin resistant in 21 days (10). Twenty-two male Sprague-Dawley rats were housed and maintained as described. After a week, they were divided into two weight-matched groups of 11 rats each. One group was offered ad libitum chow and the second group was offered the Choice Diet-chow, 30% sucrose solution (LS) (Kroger Sugar, Hood Packing, Hamlet, NC), and lard (Armour, ConAgra Foods, Omaha, NE). The bottle containing LS was placed next to the water bottle and the lard was given in a dish inside the cage with the chow.

Peripheral leptin responsiveness was tested in rats fed choice or chow on day 33. They were fasted for 10 h starting at 7.00 AM. At 5.00 PM, half of the rats in each dietary group received an intraperitoneal injection of 2 mg/kg leptin or an equivalent volume of phosphate-buffered saline (PBS). Food was returned to the cages at 6.00 PM 1 h before the start of the dark phase. Energy intakes and body weights were measured 14, 24, and 36 h after the food was returned to the cages. On day 36, the rats were tested a second time with treatments reversed. Rats that had been injected with leptin on day 33 were injected with PBS, and those that had been injected with PBS were injected with leptin. Leptin resistance was confirmed in the diet-choice rats.

The rats were fitted with jugular catheters and infusions were performed 7 and 10 days after surgery. The rats had ad libitum access to food and water until they were moved to the testing room. The average body weights of the chow rats at the time of infusion was 402 ± 8 g and choice rats body weight was 398 ± 7 g. The infusions were performed as described for experiment 1 except that baseline glucose readings before infusion were taken every 30 min to reduce stress on the rats due to frequent handling immediately after the tail cut. An additional set of infusions were performed (infusion day III) where the rats were infused only with HD leptin (3.8 μg·kg⁻¹·min⁻¹) for 16 min to determine whether the lower dose was required or if the high dose was sufficient for circulating leptin to lower serum blood glucose.

Experiment 3. In experiments 1 and 2 normal chow rats demonstrated a significant decrease in serum glucose with acute venous leptin infusions (LD followed by HD) compared with saline infusions. However, leptin-resistant, choice diet rats when infused with leptin, showed no changes in blood glucose compared with saline infusions. A number of studies testing direct and indirect leptin effects on target tissues (e.g., pancreas, liver, skeletal muscle, and brain) have been used to determine the mechanism of leptin-mediated correction of blood glucose (14, 17, 23, 29); and leptin-mediated suppression of glucagon levels has been shown to be responsible for reducing blood glucose in Type 1 diabetic mice (36). The study described here was designed first to confirm the findings from experiment 2, that the significant decrease in serum blood glucose with acute venous leptin administration is directly related to the leptin responsiveness of the rats, and second, to determine whether leptin infusion suppressed glucagon concentrations in normal rats.

We have recently reported that rats offered 30% sucrose solution in addition to chow become leptin resistant in 32 days (10). This experiment was carried out with rats offered chow plus 30% sucrose solution to test the effect of LD and HD leptin infusion in a second model of leptin resistance to establish proof of concept.

A total of 20 male Sprague-Dawley rats were housed and maintained as described as above for 1 wk. After a week, the animals were divided into two weight-matched groups of 10 rats each. One group was offered ad libitum chow and the second group was offered chow + sucrose [30% sucrose solution (wt/vol)]. The bottle containing liquid sucrose (LS) was placed next to the water bottle. Peripheral leptin responsiveness was tested on day 33 as described for experiment 2 except that there was only one test day. Therefore, half of the rats were injected with saline and half with leptin, and the leptin responsiveness was assumed to be representative of all animals within each dietary group. One week after the rats were tested for peripheral leptin responsiveness, they were fitted with venous catheters. Infusions were performed 7 and 10 days after surgery. The average body weights of the chow rats at the time of infusion was 399 ± 10 g and choice rats body weight was 405 ± 20 g. They were fasted for 10 h starting at 7.00 AM. At 7.00 PM, half of the rats in each dietary group received an intraperitoneal injection of 2 mg/kg leptin or an equivalent volume of phosphate-buffered saline (PBS). Food was returned to the cages at 6.00 PM 1 h before the start of the dark phase. Energy intakes and body weights were measured 14, 24, and 36 h after the food was returned to the cages. On day 36, the rats were tested a second time with treatments reversed. Rats that had been injected with leptin on day 33 were injected with PBS, and those that had been injected with PBS were injected with leptin. Leptin resistance was confirmed in the diet-choice rats.
Data analysis. Statistically significant differences between treatment groups were determined using Statistica software version 9.0 (StatSoft, Tulsa, OK). Differences were considered significant at \( P < 0.05 \). Single end-point measures were compared by paired t-test, one-way ANOVA or repeated measures ANOVA depending upon experimental design. Post hoc differences were determined using Duncan’s multiple-range test. For regression analysis, a Spearman coefficient of \( \geq 0.71 \) was considered to be significant at \( P < 0.01 \) for \( n = 14 \) rats, degrees of freedom = 12.

RESULTS

Experiment 1. Data from the pilot experiment with rats infused with LD leptin (1.9 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) for 22 min showed a nonsignificant decrease in blood glucose from baseline (Fig. 1). When the LD leptin infusion was immediately followed by a HD leptin (3.8 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) for another 22 min, blood glucose levels continued to decrease and were significantly lower than baseline compared with saline infusions. The inhibitory effect of leptin on blood glucose was reversed 15 min after infusion ended (Fig. 1). Rats infused with LD leptin for 16 min showed a gradual nonsignificant decrease in blood glucose from baseline that was not different from blood glucose in saline-infused rats (Fig. 2A) similar to data from the pilot experiment. When the LD leptin infusion was immediately followed by a HD leptin infusion for another 16 min, blood glucose levels continued to decrease and were significantly lower than baseline and compared with saline infusions at 28 min and had stabilized by 32 min of infusion (Fig. 2A).

The blood glucose readings at baseline were averaged over 15 min, and those during leptin infusion were averaged for the last 6 min of infusion for each dose of leptin. The data are shown in Fig. 2B and a similar representation is used for data from experiments 2 and 3. The rats showed a nonsignificant decrease in glucose from baseline with LD leptin infusion (\( P < 0.09 \)) and a significant decrease in glucose from baseline when the LD leptin infusion was followed by a HD leptin infusion (\( P < 0.008 \)). The same rats when infused with saline showed no changes in blood glucose throughout the 32 min of infusion (Fig. 2B). There was an increase in serum leptin (Table 1) but no change in serum insulin (saline infusion 0.5 ± 0.2 ng/ml; leptin infusion 0.5 ± 0.2 ng/ml) at the end of infusion. There were also no differences in weight change of the saline-infused and leptin-infused rats during the 18 h following infusion (Fig. 2C).

Table 1. Serum leptin in rats (experiments 1, 2, and 3) after saline, leptin, and high-dose leptin infusions

<table>
<thead>
<tr>
<th></th>
<th>Serum Leptin, ng/ml</th>
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<tr>
<td></td>
<td>Saline</td>
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<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>Chow rats</td>
<td>1.8 ± 0.7(^a)</td>
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<tr>
<td>Choice rats</td>
<td>2.0 ± 0.2(^a)</td>
</tr>
<tr>
<td>Sucrose rats</td>
<td>5.7 ± 0.9(^b)</td>
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<tr>
<td>Experiment 2</td>
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<tr>
<td>Chow rats</td>
<td>2.0 ± 0.2(^a)</td>
</tr>
<tr>
<td>Choice rats</td>
<td>5.7 ± 0.9(^b)</td>
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<tr>
<td>Experiment 3</td>
<td>3.7 ± 1.1(^a)</td>
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<tr>
<td>Sucrose rats</td>
<td>3.4 ± 0.9(^b)</td>
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Data are means ± SE for groups of 8–12 rats. Low-+ high-dose leptin infusion measures were made after 32 min and 16 min infusions of each dose. High-dose leptin infusion measures were made after 16 min of only high-dose leptin infusion. Values for serum leptin that do not share a common superscript letter are significantly different at \( P < 0.05 \).
Experiment 2. Food intake and body weight change following a leptin injection was used to test for leptin responsiveness in the choice and chow rats. Chow rats gained significantly less weight 14, 24, and 36 h after an intraperitoneal injection of 2 mg/kg leptin compared with an injection of saline but only showed a significant decrease in cumulative food intake 36 h after injection. Choice rats showed no changes in cumulative food intake or body weight gain 14, 24, or 36 h after an intraperitoneal injection of leptin compared with a saline injection (Fig. 3, A and B). This confirmed that the Chow rats were leptin responsive and choice rats were leptin resistant. Chow rats infused intravenously with LD leptin for 16 min followed by a 16-min infusion of HD leptin demonstrated a significant decrease in blood glucose at the end of the total infusion period compared with baseline. The rats showed no changes in blood glucose when infused with saline for the same period of time (Fig. 4A). Choice rats infused with leptin showed no changes in blood glucose after LD and HD infusions or after saline infusion (Fig. 4C). Serum leptin was increased 12- to 20-fold after 32 min of leptin infusion in both chow and choice rats (Table 1). Choice rats had a much higher serum leptin after leptin infusion compared with chow rats suggesting a slower clearance of leptin in the leptin-resistant choice rats (Table 1). Chow and choice rats infused with only the HD of leptin for 16 min showed no change in blood glucose from baseline and were not different from saline-infused rats (Fig. 4, B and D) even though serum leptin was increased 6- to 10-fold at the end of the 16-min leptin infusion (Table 1). There was no change in 18-h weight gain of either chow or choice rats following either infusion of leptin (Chow rats $-4 \pm 1$ g following leptin and $-3 \pm 1$ g following saline infusion; Choice rats $-2 \pm 1$ g following leptin and $-1 \pm 1$ g following saline infusion).

Experiment 3. Body weight and food intake change, measured 14, 24, and 36 h after a leptin injection, were used to test for leptin responsiveness in the sucrose- and chow-fed rats. Chow rats showed a significant difference in body weight change as well as a decrease in cumulative food intake 12, 24, and 36 h after injection of 2 mg/kg ip leptin compared with an injection of saline. Sucrose rats showed a cumulative increase in food intake compared with Chow rats; however, leptin injection in these rats had no effect on food intake or body weight at any time point (Table 2). This confirmed that the Chow rats were leptin responsive and sucrose rats were leptin resistant.

Chow rats infused intravenously with LD leptin for 16 min followed by a 16-min infusion of HD leptin showed a significant decrease in blood glucose at both 16 and 32 min compared with baseline. The Chow rats showed no changes in blood glucose when infused with saline for the same time (Fig. 5A). Sucrose rats infused with either saline or leptin showed no changes in blood glucose compared with baseline (Fig. 5B). Leptin infusion caused a three- to fivefold increase in serum leptin (Table 1) but had no effect on serum insulin at the end of the infusion (Chow rats saline infusion $-0.7 \pm 0.1$ ng/ml; Chow rats leptin infusion $-1.0 \pm 0.2$ ng/ml; sucrose rats saline infusion $-1.1 \pm 0.2$ ng/ml; sucrose rats leptin infusion $-1.5 \pm 0.2$ ng/ml). There were no differences in weight gain of either Chow or sucrose rats during the 18 h following infusion (Chow rats $-3 \pm 1$ g following leptin and $-2 \pm 1$ g following saline infusion; sucrose rats $-2 \pm 1$ g following leptin and $-4 \pm 1$ g following saline infusion). There was no effect of leptin infusion on plasma glucagon concentration measured at the end of the infusion in either Chow or sucrose rats (Fig. 5C).

A scatter plot analysis comparing change in 36 h food intake following leptin injection and decrease in blood glucose after leptin infusion in leptin-responsive rats from both experiments 2 and 3 indicated a strong positive linear relationship between the two variables with a Spearman correlation coefficient ($\rho = 0.87$ and $R^2 = 0.75$ ($R^2 = $ square of the Pearson correlation coefficient)) (Fig. 6A). There was no linear relationship between the two variables in leptin-resistant choice or sucrose-fed rats with correlation coefficients of $\rho = 0.31$ and $R^2 = 0.09$ (Fig. 6B).

**DISCUSSION**

Continuous subcutaneous or central administration of leptin has been shown to improve glucose homeostasis in rodent models of Type 1 and Type 2 diabetes (5, 13, 19, 35). This normalization of blood glucose levels by peripheral or central leptin administration has been established independent of changes in food intake, body weight, or circulating insulin concentrations (5, 13). The objective of the studies described here was to test whether an acute venous leptin infusion changed blood glucose in nondiabetic Sprague-Dawley rats and to determine whether this measure can be used as an acute method to differentiate between leptin-responsive and leptin-
resistant rats. The results show that systemic administration of LD leptin (1.9 μg·kg⁻¹·min⁻¹), followed by HD leptin (3.8 μg·kg⁻¹·min⁻¹) for 16 min each, results in a significant decrease in serum glucose concentrations in normal, leptin-responsive male Sprague-Dawley rats but not in leptin-resistant rats.

Leptin was originally proposed as a feedback signal in energy balance regulation (37). However, it is now implicated in influencing multiple endocrine systems including those involved in glucostasis (6). Chronic subcutaneous infusion of leptin in leptin-sufficient and leptin-responsive streptozotocin-induced Type 1 diabetic mice (STZ-T1DM) or UCD-Type 2 diabetic mice (University of California, Davis, CA) for 7–12 days has been shown to normalize blood glucose concentrations (5, 35). Similarly, central leptin injections in STZ-T1DM mice have also shown to cause a decrease in blood glucose (19). In addition, daily intraperitoneal leptin injections in hyperglycemic leptin-deficient ob/ob mice lower blood glucose (26), whereas chronic subcutaneous infusion of a leptin antagonist in wild-type mice has been shown to cause hyperglycemia, hyperinsulinemia, and leptin resistance in 3 days without changes in body weight (18). We are not aware of any papers in the literature examining the effect of leptin infusion on blood glucose in rats with normoglycemia; however, the above-mentioned studies in diabetic mice strongly suggest that leptin is essential for the maintenance of blood glucose.

The studies described here evaluated the acute effect of leptin on blood glucose regulation. Experiments 1 and 2 (Figs. 2 and 4) indicated that an acute 16-min infusion of either LD leptin or HD leptin alone is unable to change blood glucose in normal chow rats suggesting that the LD is essential to prime the rat for the HD leptin infusion. In several of the animals

Table 2. Body weight change and cumulative energy intake in sucrose and chow rats (experiment 3) after leptin injection

<table>
<thead>
<tr>
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<th>Chow Rats</th>
<th>Sucrose-Fed Rats</th>
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<tr>
<td></td>
<td>Saline intraperitoneal injection</td>
<td>Leptin (2 mg/kg) intraperitoneal injection</td>
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<tr>
<td>Body weight change, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 h</td>
<td>-14 ± 1a</td>
<td>-9 ± 1b</td>
</tr>
<tr>
<td>24 h</td>
<td>8 ± 1c</td>
<td>3 ± 1d</td>
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<tr>
<td>36 h</td>
<td>15 ± 1e</td>
<td>11 ± 1f</td>
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<tr>
<td>Cumulative energy intake, kcal</td>
<td></td>
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</tr>
<tr>
<td>14 h</td>
<td>69 ± 2a</td>
<td>57 ± 2b</td>
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<tr>
<td>24 h</td>
<td>82 ± 1c</td>
<td>70 ± 1d</td>
</tr>
<tr>
<td>36 h</td>
<td>139 ± 1f</td>
<td>126 ± 2e</td>
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Data are means ± SE for groups of 8–9 rats. The energy intake measures are cumulative over 14, 24, 36 h after intraperitoneal injection of saline or 2 mg/kg leptin. Values for a specific parameter that do not share a common superscript are significantly different at P < 0.05.
infused with only HD leptin we observed a brief drop in blood glucose that was rapidly reversed. Therefore, it is possible that HD leptin infusion alone is unable to correct blood glucose because it causes a rapid decrease in blood glucose, which is great enough to trigger compensation by other glucostatic modulators to prevent hypoglycemia. Priming the rat with the LD may be required to facilitate the mechanism involved in the leptin-mediated sustained reduction in blood glucose. By contrast, experiment 3 (Fig. 5) shows that an acute intravenous infusion of LD leptin (1.9 μg·kg⁻¹·min⁻¹) for 16 min is sufficient to cause a significant decrease in blood glucose. It has been well documented that blood glucose is very tightly regulated (16, 23, 32), and the variability in results of our experiments could be due to the differential timing of other factors such as glucagon, insulin, incretin, etc. that influence glucose homeostasis as opposed to the timing of leptin’s effect on blood glucose. Alternatively, it could indicate that there is a broad range of leptin sensitivity for glycemic control in rats and that this is influenced by the age of the rats at the time of infusion (approximate ages: experiment 3, 15 wk; experiment 1, 10 wk; experiment 2, 13 wk) or individual differences in metabolism. Another reason for the variable response to LD leptin could be differences in recovery from surgeries or in room temperature. This, however, is unlikely because all experimental conditions were maintained constant across experiments. Finally, depending on the weight of the rat, the infusion rate was set to deliver an average of 3.0–3.5 μl leptin/min for the low dose (16 min) and 6–7 μl leptin/min for the high dose (16 min). Total infusion volumes of leptin delivered (low dose: 48–56 μl; high dose: 96–112 μl) are extremely small compared with the total blood volume of 27–30 ml for a 300-g rat. The high-dose infusion volume, although double that of the low dose, is still very small compared with the total blood volume, and this should not cause an independent change in glucose due to a dilution effect. Leptin infusion increased serum leptin concentration 10-fold more in both chow and choice rats in experiment 2 than in experiments 1 and 3. Rats in experiment 2 were heavier than rats from experiment 1 at the time of infusion (402 g vs. 298 g average body weights at time of infusion) and therefore infused with more leptin based on their body weight. We speculate that this may account for the difference.

Studies have shown that regulation of glucose by leptin is independent of its effects on body weight and food intake. Daily central leptin injections (10 μg/day per rat, third ventricle) in STZ-T1DM rats caused a significant decrease in blood glucose after day 3 with no change observed in pair-fed rats. A decrease in food intake and body weight was only observed after day 6 in leptin-injected rats suggesting that leptin’s effect on lowering blood glucose is not only independent of its effect on reducing food intake but also is apparent at an earlier time point and thus is a relatively acute response (19). Similar experiments with peripheral leptin injections in T1DM and ob/ob mice have confirmed the independent regulation of glucose by leptin (30, 36). The findings from our experiments in normal rats are consistent with these studies in Type 1 diabetic rats where leptin-mediated correction of blood glucose occurs independently of changes in serum insulin and before leptin can affect food intake and body weight (35, 36). Chow rats infused with LD and HD leptin show a stable decrease in blood glucose after 32 min without changes in insulin and body weight change. Our experiments also show that leptin-resistant choice or sucrose rats are insensitive to the effects of acute intravenous leptin on lowering blood glucose.
It is important to note that when a comparison between decrease in food intake 36 h after leptin injection and decrease in blood glucose after 32 min (low + high dose) leptin infusion in leptin-responsive rats (A) and leptin-resistant rats (B) from experiments 2 and 3. A Spearman coefficient ($\rho$) $\geq$ 0.71 is significant at $P < 0.01$ for $n = 14$ rats; degrees of freedom $= 12$.

Acute intravenous leptin infusions in our experiments had no effect on glucagon levels measured at the end of the infusion in normal and sucrose rats despite emerging evidence of the inhibitory effect of leptin on glucagon secretion and circulating glucagon levels (6, 8, 35, 36). One time point measure at the end of the study may not be a good test and may be a reason why our experiments were not successful in identifying the involvement of glucagon suppression as a mechanism. Alternatively, the reduction of blood glucose may occur due to the effect of leptin on other target tissues or hormones that modulate glucose metabolism. Leptin improves insulin sensitivity and increases glucose uptake in skeletal muscle and brown adipose tissue (14). Although leptin directly inhibits insulin synthesis and secretion in $\beta$-cells in vitro and in vivo within minutes (17), it also has an inhibitory effect on secretion of corticosterone (9, 28), which could independently increase insulin sensitivity. In addition, intraperitoneal leptin administered in rodents can lower blood glucose by increasing the levels of the incretin GLP-1, a glucose-lowering hormone (1). Thus leptin has the potential to indirectly increase insulin sensitivity by acting on circulating hormones. The indirect effect of leptin on these hormones may contribute to the mechanism by which acute intravenous leptin infusions lower blood glucose and, as a result, we cannot exclude the direct effect of leptin on glucagon as a potential modulator.

There are several advantages of this method of testing leptin responsiveness, and although some are common with other current methods, when combined together they make it a more economical, easy, and quick way to test leptin sensitivity in rats. First, the experiment is very economical, requiring the use of very little leptin per rat (90 $\mu$g/kg) compared with measuring food intake (2 mg/kg). Second, this acute test requires a total of 4 days including the day of surgery, but once the experiment is set up it requires only 2 h of measurement. Measurement of food intake after leptin injection requires 2–3 days of baseline food intake before the leptin injection and up to 3 days of food intake measurements after injection, and therefore the two are comparable in terms of the total time involved. Third, the rats are not killed for the test and can be used for an experiment. Fourth, although the test requires the rats to be catheterized, this is a less aggressive surgery than would be required for cannula placement if central leptin responsiveness was being tested. Finally, experiments exploring the effects of leptin often involve catheter placements for intravenous infusions of leptin (27, 34). It is advantageous to use this method in such experimental conditions as it will reduce the time taken to determine leptin responsiveness in the animal. There are also some limitations of the method. First, any stress inflicted on the rats will cause hyperglycemia. In experiments 2 and 3 we reduced the frequency of baseline sampling to once every 30 min to minimize this problem. Second, there appears to be a large range of leptin sensitivity for leptin’s effect on lowering blood glucose where low-dose leptin may or may not be sufficient to reduce blood glucose and as a result, a combination of both low- and high-dose leptin is required to be infused to ensure that leptin responsiveness is measured accurately. Finally, this method like other current methods requires testing a group of rats to determine leptin responsiveness, and although some are common with other current methods, when combined together they make it a more economical, easy, and quick way to test leptin sensitivity in rats. First, the experiment is very economical, requiring the use of very little leptin per rat (90 $\mu$g/kg) compared with measuring food intake (2 mg/kg). Second, this acute test requires a total of 4 days including the day of surgery, but once the experiment is set up it requires only 2 h of measurement. Measurement of food intake after leptin injection requires 2–3 days of baseline food intake before the leptin injection and up to 3 days of food intake measurements after injection, and therefore the two are comparable in terms of the total time involved. Third, the rats are not killed for the test and can be used for an experiment. Fourth, although the test requires the rats to be catheterized, this is a less aggressive surgery than would be required for cannula placement if central leptin responsiveness was being tested. Finally, experiments exploring the effects of leptin often involve catheter placements for intravenous infusions of leptin (27, 34). It is advantageous to use this method in such experimental conditions as it will reduce the time taken to determine leptin responsiveness in the animal. There are also some limitations of the method. First, any stress inflicted on the rats will cause hyperglycemia. In experiments 2 and 3 we reduced the frequency of baseline sampling to once every 30 min to minimize this problem. Second, there appears to be a large range of leptin sensitivity for leptin’s effect on lowering blood glucose where low-dose leptin may or may not be sufficient to reduce blood glucose and as a result, a combination of both low- and high-dose leptin is required to be infused to ensure that leptin responsiveness is measured accurately. Finally, this method like other current methods requires testing a group of rats to determine leptin responsiveness.
responsiveness. Future studies will involve testing female rats to determine whether there are any differences in acute leptin responsiveness between sexes and to extend the method to all rats.

**Perspectives and Significance**

In summary, we can conclude that systemic administration of low-dose leptin (1.9 μg·kg^{−1}·min^{−1}), followed by high-dose leptin (3.8 μg·kg^{−1}·min^{−1}) for 16 min each, results in a significant decrease in serum glucose concentrations in normal leptin-responsive rats but not in leptin-resistant rats. Although our studies show no changes in circulating glucagon concentration, we have not excluded the suppression of glucagon as a potential mechanism for the effect based on the fact that we only tested glucagon as an end point measure. In addition, other leptin-mediated modulators in the system may contribute to this response. While the studies described here did not investigate the mechanism of leptin action, the effect was consistent across different groups of leptin-responsive rats with a very small variability but was absent in leptin-resistant rats and can be used as an acute method to test leptin responsiveness in rats.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: B.N.D. and R.B.H. performed experiments; B.N.D. interpreted results of experiments; B.N.D. prepared analyzed data; B.N.D. interpreted results of experiments; B.N.D. drafted manuscript; B.N.D. edited and revised manuscript; R.B.H. conceived of and designed research; R.B.H. approved final version of manuscript.

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