4-2012

Effects of leptin replacement alone and with exendin-4 on food intake and weight regain in weight-reduced diet-induced obese rats

Roger Reidelberger
Veterans Affairs Nebraska-Western Iowa Health Care System

Alvin Haver
Veterans Affairs Nebraska-Western Iowa Health Care System

Prasanth K. Chelikani
Creighton University

Bettye Apenteng
Creighton University

Curtis Perriotte-Olson
Veterans Affairs Nebraska-Western Iowa Health Care System

See next page for additional authors

Authors
Roger Reidelberger, Alvin Haver, Prasanth K. Chelikani, Bettye Apenteng, Curtis Perriotte-Olson, Krista Anders, Sharalyn Steenson, and James E. Blevins

Am J Physiol Endocrinol Metab 302: E1576–E1585, 2012.
First published April 17, 2012; doi:10.1152/ajpendo.00058.2012.

Effects of leptin replacement alone and with exendin-4 on food intake and weight regain in weight-reduced diet-induced obese rats

Roger Reidelberger,[1,2] Alvin Haver,[1,2] Prasanth K. Chelikani,[2,5] Bettye Apenteng,[2] Curtis Perriotte-Olson,[1] Krista Anders,[2] Sharalyn Steenson,[2] and James E. Blevins[3,4]

[1]*Veterans Affairs Research Service, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, Nebraska;* [2]*Department of Biomedical Sciences, Creighton University, Omaha, Nebraska;* [3]*Veterans Affairs Research Service, Veterans Affairs Puget Sound Health Care System, Seattle, Washington;* [4]*Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington School of Medicine, Seattle, Washington; and* [5]*Gastrointestinal Research Group, Snyder Institute for Chronic Diseases, Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada*

Submitted 2 February 2012; accepted in final form 17 April 2012

Reidelberger R, Haver A, Chelikani PK, Apenteng B, Perriotte-Olson C, Anders K, Steenson S, Blevins JE. Effects of leptin replacement alone and with exendin-4 on food intake and weight regain in weight-reduced diet-induced obese rats. *Am J Physiol Endocrinol Metab* 302: E1576–E1585, 2012. First published April 17, 2012; doi:10.1152/ajpendo.00058.2012.—Weight loss in obese humans produces a relative leptin deficiency, which is postulated to activate potent orexigenic and energy conservation mechanisms to restrict weight loss and promote weight regain. Here we determined whether leptin replacement alone or with GLP-1 receptor agonist exendin-4 attenuates weight regain or promotes greater weight loss in weight-reduced diet-induced obese (DIO) rats. Forty percent restriction in daily intake of a high-fat diet in DIO rats for 4 wk reduced body weight by 12%, body fat by 29%, and plasma leptin by 67% and normalized leptin sensitivity. When food restriction ended, body weight, body fat, and plasma leptin increased rapidly. Daily administration of leptin [3-h intraperitoneal (ip) infusions (4 nmol·kg^{-1}·h^{-1})] at onset and end of dark period for 3 wk did not attenuate hyperphagia and weight regain, nor did it affect mean daily meal sizes or meal numbers. Exendin-4 (50 pmol·kg^{-1}·h^{-1}) infusions during the same intervals prevented postrestriction hyperphagia and weight regain by normalizing meal size. Coadministration of leptin and exendin-4 did not reduce body weight more than exendin-4 alone. Instead, leptin began to attenuate the inhibitory effects of exendin-4 on food intake, meal size, and weight regain by the end of the second week of administration. Plasma leptin in rats receiving leptin was sevenfold greater than in rats receiving vehicle and 17-fold greater than in rats receiving exendin-4. Together, these results do not support the hypothesis that leptin replacement alone or with exendin-4 attenuates weight regain or promotes greater weight loss in weight-reduced DIO rats.

food restriction; hyperphagia; leptin sensitivity; glucagon-like peptide-1 receptor agonist

OBESITY IS A CHRONIC, stigmatized, and costly disease that is rarely curable and is increasing in prevalence in most of the world (4). Current therapies for producing weight loss in obese individuals, i.e., dieting, exercise, and medications, are woefully ineffective in producing long-term weight loss. This is likely due to redundancy and plasticity in the complex physiological system that controls food intake and regulates energy reserves. Many experts believe that multidrug therapy aimed at different components of this regulatory system will be required to produce a significant reduction in adiposity (4, 13, 21, 22).

An important early step in the development of antiobesity drugs is determining whether chronic administration of anorexigenic substances, alone or in combination, can produce a prolonged decrease in daily food intake and adiposity in experimental animals. Methods of administration usually include daily injections or insertion of an osmotic minipump beneath the skin or into the peritoneal cavity to deliver substances continuously for 1 wk or more. Treatments typically produce only transient decreases in food intake, resulting in relatively small or no decreases in body weight and adiposity (e.g., see Refs. 16, 28, 38, 43, and 44). Likely reasons include development of a compensatory increase in food intake between injections, desensitization and downregulation of receptors in response to continuous or frequent administration of high doses, and weight loss-induced activation of counteracting orexigenic and energy conservation mechanisms to restore energy reserves (14, 24, 37, 39).

We have developed a novel experimental model that permits precise control of intravenous (iv) or intraperitoneal (ip) administration of anorexigenic substances to rats tethered via infusion swivels to computer-controlled pumps (7–10, 30–32, 34). Rats are free to move, eat, and drink within their home cages, and their indwelling catheters remain functional for many months. Measurement of food bowl weight, recorded by computer every 20 s, permits daily assessment of the instantaneous effects of dose and pattern of administration of substances on food intake and meal patterns. Adjustments in dosing regimen can be performed daily within rats in an attempt to define a strategy that minimizes compensatory hyperphagia between doses, receptor desensitization and downregulation, and activation of counteracting orexigenic mechanisms.

We used this experimental model to help resolve an international controversy among many scientists from academia and industry on whether the gut hormone peptide YY (3–36) [PYY(3–36)] reduces food intake and body weight (2, 7, 9, 15, 27, 43). We demonstrated that 3-h iv infusion of PYY(3–36), which is more likely to simulate postprandial secretion of the gut hormone, dose-dependently reduces short-term food intake in rats and that daily intermittent PYY(3–36) infusions reduce body weight in lean and diet-induced obese (DIO) rats (7, 9, 11). We concluded that, because of its short half-life, bolus systemic injection of PYY(3–36) to rodents, the method used

Address for reprint requests and other correspondence: R. Reidelberger, VA-NWIHCS (151), 4101 Woolworth Ave., Omaha, NE 68105 (e-mail: rogerr@creighton.edu).

routinely by most investigators to study mechanisms of action of putative satiety hormones, does not reliably reduce food intake and body weight. We also concluded that continuous subcutaneous(SQ) infusion of PYY(3–36), unlike intermittent systemic infusion, was ineffective in reducing body weight in the prior studies because it probably produced desensitization and downregulation of receptors for PYY(3–36). Others have argued that bolus ip injections can produce acute stress and that stress can mask the inhibitory effect of anorexigenic substances on food intake (1). This is not a factor with our experimental model.

Recently, we have used our experimental model to determine the effects of a wide range of dosing regimens of PYY(3–36), amylin, amylin receptor agonist salmon calcitonin, naltrexone, and glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 on daily food intake, body weight, and adiposity in DIO rats (10, 11, 29, 31–33). Of these substances, only a single dosing regimen of a single substance (exendin-4) was able to produce a sustained ~15–30% reduction in daily food intake and weight loss for >2 wk (31). However, exendin-4's effect waned after 17 days, and coadministration of PYY(3–36) and exendin-4 at various dosing combinations did not produce a more prolonged effect than the GLP-1 receptor agonist exendin-4 alone despite recent studies suggesting that chronic elevation in gut hormones PYY(3–36) and GLP-1 may cause the significant weight loss that occurs with Roux-en-Y gastric bypass surgery (19, 20).

In each of our studies, the gradual decline in efficacy of the various treatments to reduce food intake and body weight in the DIO rats did not appear to be due to desensitization or downregulation of receptors (10, 11, 29, 31–33). This is because food intake increased significantly when treatments were discontinued for 1 day following apparent losses in treatment efficacies. If desensitization or downregulation of receptors was primarily responsible for loss in a treatment efficacy, then discontinuing the treatment should have had little, if any, effect on food intake. Rather, our results suggest that activation of potent orexigenic mechanisms occurred gradually to counteract the inhibitory effect of the anorexigenic substances on food intake and energy reserves. The nature of these mechanisms remains to be determined. One possibility is that early treatment-induced reductions in daily food intake and energy reserves elicit a delayed compensatory response to restore energy balance, which is mediated by a reduction in leptin signaling to the brain (24, 37). In support of this hypothesis, Rosenbaum et al. (37) have shown that a 10% weight reduction in obese humans reduces energy expenditure, satiation, and perception of amount of food eaten and that low-dose leptin replacement reverses these effects (36, 37).

Here, we determined whether leptin replacement alone or with GLP-1 receptor agonist exendin-4 attenuates weight regain or promotes greater weight loss in weight-reduced, DIO rats provided free access to a high-fat diet. We first determined the effects of food restriction-induced weight loss in DIO rats on food intake, body weight, body fat content, and plasma leptin. We next identified a leptin-dosing regimen that would produce a sustained reduction in food intake and body weight in a lean rat model. We then determined the effects of intermittent ip infusion of this dosing regimen of leptin alone and in combination with exendin-4 on hyperphagia and weight regain in weight-reduced DIO rats. We then assessed whether the inability of this leptin treatment to prevent weight regain or promote greater weight loss in the weight-reduced DIO rats was due to a relative leptin insensitivity in these rats.

METHODS

Exendin-4 and Leptin

Exendin-4 was synthesized and purified as described previously (31). Recombinant mouse leptin was obtained from Dr. A. F. Parlow, National Hormones and Peptides Program, Harbor-UCLA Medical Center, Los Angeles, CA. Leptin was dissolved at 50 nmol/ml in phosphate-buffered saline (PBS) at pH 8. One-milliliter aliquots were then stored at −70°C until they were used.

Animals

The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the protocol. Outbred male lean (Sprague-Dawley; Charles River Laboratories) and DIO rats (CD IGS; Charles River Laboratories) were used in the experiments. There appears to be no consensus in defining obesity in rats, although percent body fat >20% body weight is likely (25). Here, we induced obesity (≥25% body fat), as described previously (31), with a 45% fat diet (D12451; Research Diets, New Brunswick, NJ) over a 4- to 6-mo period. Total body fat mass was determined noninvasively using quantitative magnetic resonance (EchoMRI 700; Echo Medical Systems, Houston, TX).

Effects of Food Restriction-Induced Weight Loss in DIO Rats on Food Intake, Body Weight, Body Fat, and Plasma Leptin

This experiment assessed whether food restriction-induced weight loss in DIO rats produces a post-restriction-induced hyperphagia and weight regain that could possibly be caused by a relative leptin deficiency. Sixty DIO rats were divided into three groups of 20 rats each with matched body weights (858 ± 21, 853 ± 13, and 852 ± 19 g) and fat masses (271 ± 16, 262 ± 11, and 262 ± 13 g). One group was provided free access to the 45% fat diet for 7 wk. In the other two groups of rats, a 40% reduction in the daily intake of the high-fat diet was imposed for 2 or 4 wk, followed by 3 wk of free access to the high-fat diet. Restricted rats were fed 60% of the amount of food consumed by the ad libitum fed group on the previous day. In 10 rats of each group, weights of food bowls were recorded by computer to determine hourly cumulative intake for each rat; in the remaining rats, food bowls were weighed at the start and end of each day. Body weights were measured weekly. Body fat contents were determined, and blood samples were taken in groups of rats at the end of intervals of food restriction and refeeding (Fig. 1). Rats were anesthetized by isoflurane inhalation, and blood samples were taken by cardiac puncture for measurement of plasma leptin concentration (Leptin ELISA, EZML-82K; Millipore, Billerica, MA).

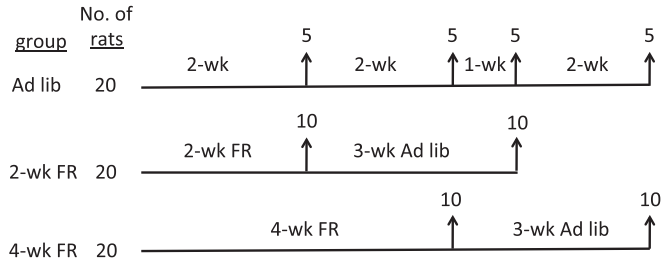

Fig. 1. Timeline of experiment examining the effects of 40% daily food restriction (FR) for 2 or 4 wk, followed by 3 wk of ad libitum (ad lib) feeding, on body weight, body fat, plasma leptin concentration, and daily food intake in diet-induced obese (DIO) rats. Arrows denote time and no. of rats euthanized for blood sampling and measurement of body fat.

Effects of Daily ip Infusions of Leptin and Exendin-4 Alone and Together on Post-Food Restriction-Induced Hyperphagia, Weight Regain, and Plasma Leptin in DIO Rats

If the hyperphagia and weight regain that follow prolonged food restriction and weight loss in DIO rats are due in part to a reduction in leptin signaling to the brain, then administration of leptin alone or with the anorexigenic peptide exendin-4 should attenuate postrestriction weight regain or produce greater weight loss.

Results from a series of preliminary experiments examining the effects of leptin administration in lean rats were used to define a leptin dosing regimen for this experiment in DIO rats. We first determined the dose response effects of a single, 3-h iv infusion of leptin (2, 4, 6, and 12 nmol·kg^{-1}·h^{-1}) at dark onset on food intake during the dark period in lean rats. We then determined the effects of two 3-h iv infusions of leptin at the minimal effective dose of 4 nmol·kg^{-1}·h^{-1} at the onset of the dark and light periods each day for 10 days on daily food intake, body weight, and body fat in lean rats. We then compared the effects of a 3-h iv vs. ip infusion of leptin (4 and 6 nmol·kg^{-1}·h^{-1}) at dark onset on food intake in lean rats, since the next longer experiment with DIO rats would administer leptin daily by ip rather than iv infusion because of the relatively limited long-term patency of iv catheters. We then determined the effects of twice daily 3-h ip infusions of leptin (4 nmol·kg^{-1}·h^{-1}) and exendin-4 (50 pmol·kg^{-1}·h^{-1}) alone and in combination for 3 wk on food intake, body weight, body fat, and plasma leptin in weight-reduced DIO rats.

Dose response effects of iv infusion of leptin on food intake in lean rats. Male rats (310–430 g) were housed individually in hanging wire mesh cages in a room with controlled temperature (19–21°C) and a 12:12-h light-dark cycle. Rats were provided pelleted or powdered rat chow (Labdiet, 5001 Rodent diet; PMI Nutrition International, Brentwood, MO) and water ad libitum. The procedure for implantation of a jugular vein catheter for peptide infusions has been described previously (45). Catheters were kept patent by flushing with 0.2 ml of heparinized saline (20 U/ml) on alternate days and plugged with stainless-steel wire. The animals were allowed ≥1 wk to recover from surgery. Rats were then adapted to being chronically tethered to an infusion swivel (Instech Laboratories, Plymouth Meeting, PA) for ≥1 wk before the start of the experiments. The jugular vein catheter was connected to a 40-cm length of tubing passed through a protective spring coil connected between a lightweight saddle worn by the rat and the swivel. Rats had ad libitum access to ground chow that was provided fresh each day 3 h before dark onset. In a series of four experiments, rats (n = 16/experiment) received a single, 3-h jugular vein infusion of leptin at 2, 4, 6, and 12 nmol·kg^{-1}·h^{-1} in 0.15 M NaCl and 0.1% BSA at 3 ml/h via a syringe infusion pump (PHD2000, Harvard Apparatus, South Natick, MA) beginning 15 min before dark onset; pumps were turned on and off by computer. In each experiment, each of the 16 rats received vehicle and a single dose of leptin in counterbalanced order at 72-h intervals. Food intake cumulated hourly for 12 h after dark onset was determined from continuous computer recording of changes in food bowl weight. At the end of an experiment, data from a rat were excluded if its jugular vein catheter was not patent. A catheter was deemed patent if the rat lost consciousness within 10 s of a bolus injection of the short-acting anesthetic Brevital (1 mg in 0.5 ml of saline) into the catheter.

Effects of daily iv infusions of leptin on food intake and body weight in lean rats. Thirty-two male rats implanted with iv catheters and tethered to infusion swivels were provided powdered rat chow from 1100 to 0900 the next morning (dark period: 2100–900). Setup and routine maintenance were performed each day from 0900 to 1100. During a 7-day baseline, all rats received two daily iv infusions of vehicle (0.15 M NaCl, 0.1% BSA, 1 ml/h) from 1100 to 1400 and 2100 to 2400. Animals were then divided into two groups, one to receive vehicle (n = 16) and the other leptin (n = 16), matched for body weight (417 ± 8 vs. 414 ± 8 g) at end of baseline. During the next 10 days, rats received two daily infusions of vehicle or leptin (4 nmol·kg^{-1}·h^{-1}) from 1100 to 1400 and 2100 to 2400. This leptin dose was the minimal effective dose that inhibited food intake in the previous experiment determining the dose response effects of iv infusion of leptin on food intake in lean rats. Daily food intake was determined from continuous computer recording of changes in food bowl weight. Rats were weighed again at end of the 10-day period, and body fat was measured. Data from a rat were excluded if its jugular vein catheter was determined to not be patent.

Effects of iv vs. ip infusion of leptin on food intake in lean rats. Methods were identical to those described in the preceding experiment determining the dose-dependent effects of iv infusion of leptin on food intake, except that each rat (n = 16, 370–520 g) was implanted with an ip as well as an iv catheter, as described previously (31). In the first experiment, rats randomly received ip infusion of vehicle, ip infusion of leptin at 4 nmol·kg^{-1}·h^{-1}, and iv infusion of leptin at 4 nmol·kg^{-1}·h^{-1} on days separated by ≥48 h. In a subsequent experiment, the same rats received vehicle and iv and ip infusions of leptin at 6 nmol·kg^{-1}·h^{-1}.

Effects of daily ip infusions of leptin and exendin-4 alone and together on post-food restriction-induced hyperphagia, weight regain, and plasma leptin in DIO rats. Intraperitoneal catheters were implanted as described previously (31). One hundred twenty-six DIO rats with ip catheters tethered to infusion swivels were divided into six groups of 21 rats each with matched body weights (740 ± 11, 730 ± 14, 722 ± 16, 735 ± 14, 724 ± 20, and 734 ± 18 g) and fat masses (181 ± 10, 194 ± 12, 167 ± 10, 187 ± 10, 171 ± 10, and 175 ± 8 g). One group was provided free access to the 45% fat diet for 7 wk. The other five groups were food restricted for 4 wk to 60% of the amount of food consumed by the ad libitum-fed group on the previous day. During the next 5 days, the food-restricted rats continued to be restricted while receiving on each day two 3-h ip infusions of 0.9% saline and 0.1% BSA from 1100 to 1400 and 2000 to 2300 (dark period from 1100 to 2300). To define a daily exendin-4 dosing strategy that reduces daily food intake in ad libitum-fed DIO rats by ~40%, the ad libitum-fed rats received 60, 20, 15, 0, and 30 pmol/h of exendin-4 during the same periods on the 5 consecutive days, preceded by a loading dose that was one-third of the hourly dose. The 30 pmol/h dosing rate (50 pmol·kg^{-1}·h^{-1}) reduced daily food intake by 38%.

During the next 3 wk, the group of rats that had been fed ad libitum (*group 1*) continued to be fed ad libitum while receiving two daily infusions of vehicle from 1100 to 1400 and 2000 to 2300. During this same 3-wk period, four of the five groups of rats that had been food restricted were fed ad libitum and were infused daily during the same periods with vehicle (*group 2*), exendin-4 [*group 3*; 30 pmol/h (50 pmol·kg^{-1}·h^{-1})], leptin [*group 4*; 2,200 pmol/h (4 nmol·kg^{-1}·h^{-1})], and exendin-4 plus leptin at these doses (*group 5*). Rats in the remaining group that had been food restricted (*group 6*) were pair-fed daily the mean amount of food ingested by the exendin-4-treated group on the previous day. Daily food intake was determined from continuous computer recording of changes in food bowl weight or by measuring the weight of food bowls at the beginning and end of each day. Meal parameters (meal size and no. of meals) were determined using a minimum meal size criterion of 50 mg and a minimum intermeal interval criterion of 15 min, as recommended by Zorrilla et al. (49). Rats were weighed weekly. At the end of the 3-wk treatment period, some rats from each group received their 3-h treatments at dark onset without food present and were then anesthetized, and blood samples were taken by cardiac puncture for measurement of plasma leptin concentration.

DIO rats in this study were provided only a calorically dense high-fat diet. Most obese people trying to lose weight also try to avoid eating palatable calorically dense foods, but most people ultimately fail to sustain this effort and regain lost weight. Pharmacotherapies that can reduce the strong drive to eat palatable calorically dense foods after weight loss are likely to be more successful in promoting and sustaining weight loss. For several years now we have been using

this DIO rat model in an attempt to identify a pharmacotherapy that will reduce consumption of a palatable high-fat food.

Leptin Sensitivity in Age-Matched Lean, DIO, and Weight-Reduced DIO Rats

Leptin sensitivity is reduced in obese subjects, and weight loss in obese subjects increases leptin sensitivity (24). We assessed whether the inability of leptin replacement to prevent weight regain in our weight-reduced DIO rats was due to a relative leptin insensitivity in these rats. Detection of an increase in leptin receptor signaling protein, phosphorylated signal transducer and activator of transcription 3 (pSTAT3), in the arcuate nucleus (ARC) by immunostaining has become the gold standard for assessing leptin sensitivity, because ARC pSTAT3 increases rapidly with stimulation of leptin receptor and because increases in ARC pSTAT3 are usually attributable to leptin action (12, 24, 35). We measured leptin sensitivity in *1*) age-matched lean rats (619 ± 9 g body wt, 11.9 ± 0.3% body fat; $n = 6$), *2*) DIO rats (965 ± 78 g body wt, 30 ± 1% body fat; $n = 5$), and *3*) DIO rats (initially 955 ± 41 g body wt, 29 ± 1% body fat; $n = 5$) after a weight loss of 10.3 ± 0.7% to 856 ± 33 g body wt was induced. Weight loss in DIO rats was induced by restricting daily intake of their 45% fat diet by 40% for 4 wk.

Rats from each group had food removed at light onset and received an ip injection of leptin (125 nmol/kg) 4–6 h later. Thirty minutes after injection, rats were anesthetized with isoflurane and then immediately perfused transcardially with cold saline then 4% paraformaldehyde in PBS. Peak expression of ARC pSTAT3 occured 30 min after leptin injection (17). Brains were placed in 4% paraformaldehyde-PBS for 24 h and then placed in 70% ethanol (EtOH) prior to paraffin embedding. Coronal sections (6 μm) sampled at 120-μm intervals through the ARC [bregma −3.48 to −2.76 mm; (26)] were obtained using a rotary microtome, slide-mounted using a floatation water bath (37°C), and baked for 30 min at 60°C to give 40 slides/brain with two sections/slide. Sections were deparaffinized in Leica Bond dewax solution (Leica Microsystems, Buffalo Grove, IL) and rehydrated through 95% EtOH. Immunohistochemical staining of pSTAT3 was performed using a modification of previously published methods (23, 46), using a Leica Bond-MAX automated immunostainer (Leica Microsystems). An antigen retrieval (heat-induced epitope retrieval) technique was used in which slides were incubated in citrate buffer (pH 6.0, Bond Epitope Retrieval Solution 1; Leica Microsystems) at 100°C for 10 min. Endogenous peroxidase activity was blocked for 5 min using 3% H_2O_2, followed by blocking in 10% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in Tris-buffered saline for 20 min at room temperature. Slides were incubated with a primary rabbit anti-pSTAT3 antibody (1:1,000; Sigma-Aldrich, St. Louis, MO) or normal rabbit IgG isotype control (1:1,000; R & D Systems, Minneapolis, MN), both in Bond Primary Antibody Diluent (Leica Microsystems), for 30 min at room temperature. Sections were incubated with goat anti-rabbit polyhorseradish peroxidase polymer secondary detection (Leica Microsystems) for 8 min at room temperature. Sections were then incubated with 3,3′-diaminobenzidine chromagen substrate (Bond Mixed Refine 3,3′-diaminobenzidine substrate detection; Leica Microsystems) for 10 min at room temperature. After being washed with dH_20, the sections were counterstained with Mayer Hematoxylin solution (Newcomer Supply, Middleton, WI), dehydrated through 100% EtOH, cleared in xylene, mounted with synthetic resin mounting medium, and coverslipped.

Slides were analyzed using bright field on a Nikon 80i microscope (Nikon Instruments, Melville, NY) and images obtained using NIS-Elements and Nikon Digital Sight Series color camera (DS-Fi1; Nikon Instruments). All measurements were made using a ×20 objective lens. Digital RGB images were exported to Photoshop (Adobe, Tucson, AZ). A neuron was considered labeled if the pSTAT3 (+) nucleus was clearly labeled. For each brain, all pSTAT3 (+) neurons were counted bilaterally in adjacent sections at four levels of ARC (bregma −3.48 to −2.76 mm). Values for each brain within a treatment were averaged to obtain the mean of the treatment group.

Statistical Analyses

Values are presented as group means ± SE. Data were analyzed by repeated-measures analysis of variance. Planned comparisons of treatment means were evaluated by *t*-tests and paired *t*-tests. Differences were considered significant if $P < 0.05$.

RESULTS

Effects of Food Restriction-Induced Weight Loss in DIO Rats on Food Intake, Body Weight, Body Fat, and Plasma Leptin

A 40% reduction in daily intake of a 45% fat diet was imposed for 2 or 4 wk in DIO rats, followed by 3 wk of free access to the high-fat diet. After 2- and 4-wk food restriction, body weight was reduced by 7 and 12% (Fig. 2*A*) and body fat by 19 and 29%, respectively (Fig. 2*B*). Unrestricted DIO rats were hyperleptinemic; after 2- and 4-wk restriction, plasma leptin was reduced by 56 and 67%, respectively (Fig. 2*C*). [Leptin levels in the unrestricted DIO rats are characterized here as hyperleptinemic because in a separate experiment we measured leptin levels of 22.9 ± 2.4 ng/ml in unrestricted DIO rats (699 ± 17 g, 28 ± 1% body fat; $n = 10$) and 5.3 ± 1.2 ng/ml in age-matched lean rats (517 ± 16 g, 16 ± 2% body fat; $n = 10$) (unpublished data).] Prolonged hyperphagia occurred after 4 wk, but not 2 wk, of caloric restriction in the DIO rats (Fig. 2*D*). After 3 wk of ad libitum refeeding, weight and body fat regain were complete in both restricted groups (Fig. 2, *A* and *B*), and plasma leptin rebounded completely after 2 wk, but not 4 wk, of restriction (Fig. 2*C*). These results demonstrate that food restriction-induced weight loss (and fat loss) in our DIO rats reverses hyperleptinemia and induces a postrestriction hyperphagia and weight (and fat) regain. This finding is consistent with the hypothesis that weight loss in obese subjects produces a relative leptin deficiency that activates orexigenic and energy conservation mechanisms in the brain to restore energy reserves.

Effects of Daily ip Infusions of Leptin and Exendin-4 Alone and Together on Post-Food Restriction-Induced Hyperphagia, Weight Regain, and Plasma Leptin in DIO Rats

Dose response effects of iv infusion of leptin on food intake in lean rats. To define a leptin-dosing regimen for the weight-reduced DIO rats, we first determined the dose response effects of a 3-h iv infusion of leptin (2, 4, 6, and 12 nmol·kg^{-1}·h^{-1}) at dark onset on food intake in lean rats. Leptin infusion dose-dependently reduced food intake in lean rats (Figs. 3, *A–D*); the minimal effective dose of 4 nmol·kg^{-1}·h^{-1} significantly decreased 3- and 12-h cumulative intakes by 26 and 21%, respectively. Leptin doses of 6 and 12 nmol·kg^{-1}·h^{-1} reduced food intake in a similar manner (Fig. 3, *C* and *D*).

Effects of daily iv infusions of leptin on food intake and body weight in lean rats. We next determined the effects of twice daily 3-h iv infusions of the minimal effective dose of leptin (4 nmol·kg^{-1}·h^{-1}) at the onset of the light and dark periods for 10 days on daily food intake, body weight, and body fat in lean rats. Leptin produced a sustained 8–22% reduction in daily

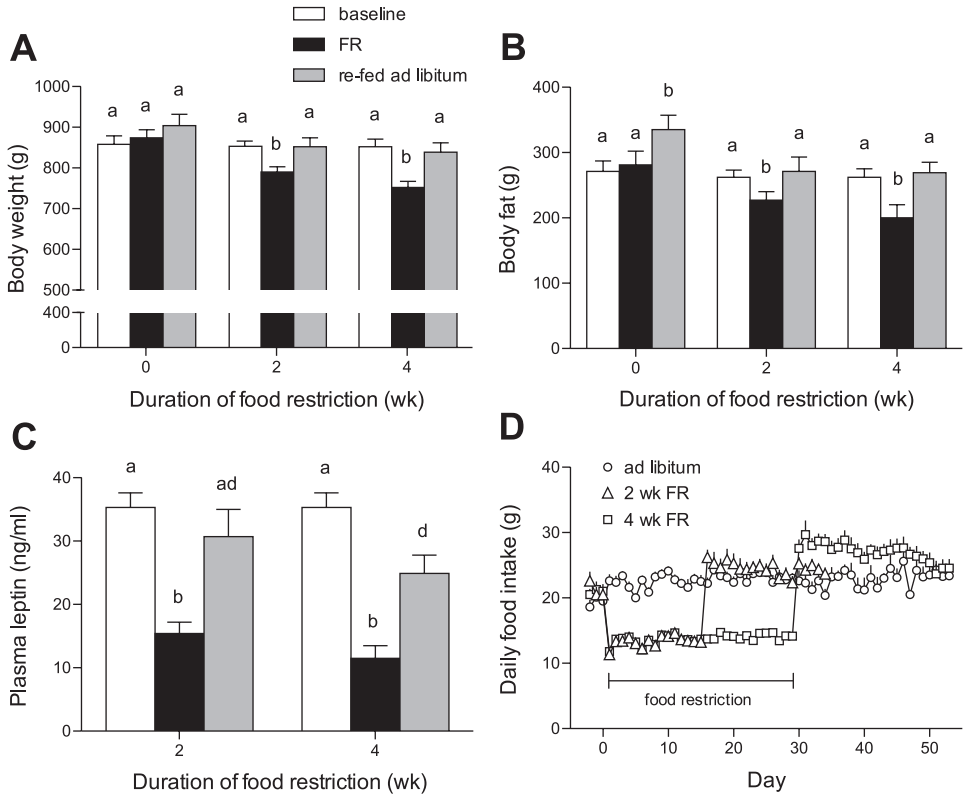

Fig. 2. Body weight (*A*), body fat (*B*), plasma leptin concentration (*C*), and daily food intake (*D*) in DIO rats in response to 40% daily FR for 2 or 4 wk, followed by 3 wk of ad libitum feeding. Values are means ± SE. Values labeled with different letters are different ($P < 0.05$).

food intake for 10 days beginning on the 1st day of administration (Fig. 3*G*) and reduced body weight and body fat by 6 and 53%, respectively, without lean mass being affected (data not shown).

Effects of iv vs. ip infusion of leptin on food intake in lean rats. We then compared the effects of a 3-h iv vs. ip infusion of leptin at dark onset on food intake in lean rats, since the next experiment determining the effects of leptin replacement for 3 wk on weight regain in weight-reduced DIO rats would administer leptin by ip infusion. At each dose, leptin reduced food intake similarly whether administered by iv or ip infusion (Fig. 3, *E* and *F*).

Effects of daily ip infusions of leptin and exendin-4 alone and together on post-food restriction-induced hyperphagia, weight regain, and plasma leptin in DIO rats. The 40% daily food restriction for 4 wk produced a 10% weight loss in DIO rats (Fig. 4*A*). During the subsequent 3-wk ad libitum feeding period, the weight-reduced rats receiving daily vehicle infusions (two 3-h ip infusions at the onset and end of the dark period) were hyperphagic for 2 wk (Fig. 5*A*) and regained lost weight (Fig. 4*A*). Hyperphagia occurred primarily through an increase in meal size (Fig. 5*B*). Compared with these rats, weight-reduced rats receiving leptin infusion (4 nmol·kg^{-1}·h^{-1}) during the same two 3-h intervals were similarly hyperphagic, had similar mean daily meal sizes and meal numbers, and regained weight at the same rate (Figs. 4*A* and 5, *A*–*C*). In contrast, the two 3-h infusions of exendin-4 (50 pmol·kg^{-1}·h^{-1}) prevented postrestriction hyperphagia and weight regain by normalizing meal size (Figs. 4*A* and 5, *A* and *B*). Prevention of weight gain was identical in rats pair-fed the daily amount of food ingested by the exendin-4-treated group (Fig. 4*A*), suggesting that exendin-4 prevented weight regain by preventing hyperphagia. Coadministration of leptin and exendin-4 did not reduce body weight more than exendin-4 alone (Fig. 4*A*). Instead, leptin began to attenuate the inhibitory effect of exendin-4 on food intake, meal size, and weight regain by the end of the 2nd wk of administration (Figs. 4*A* and 5, *A* and *B*). Our leptin dosing regimen produced hyperleptinemia in the weight-reduced DIO rats (Fig. 4*B*). Plasma leptin levels in animals receiving leptin infusions were sevenfold greater than those in rats receiving vehicle infusions and 17-fold greater than those in rats receiving exendin-4 infusions.

Leptin Sensitivity in Age-Matched Lean, DIO, and Weight-Reduced DIO Rats

ARC pSTAT3 signaling 30 min after leptin injection (125 nmol/kg ip) was reduced significantly in DIO vs. lean rats [70 ± 15 vs. 370 ± 107 pSTAT (+) cells, $P < 0.05$] and in DIO vs. weight-reduced DIO rats [70 ± 15 vs. 300 ± 33 pSTAT (+) cells, $P < 0.001$]. In contrast, pSTAT3 signaling was not different in weight-reduced DIO vs. age-matched lean rats [300 ± 33 vs. 370 ± 107 pSTAT3 (+) cells, $P > 0.05$]. Figure 6 shows representative ARC sections. pSTAT3 (+) cells were counted bilaterally from single sections at four levels of ARC [bregma −3.48 to −2.76 mm (26)]. Together, these results suggest that our DIO rats were hyperleptinemic and leptin insensitive and that a food restriction-induced weight loss of 10% in the DIO rats reversed hyperleptinemia and normalized leptin sensitivity.

DISCUSSION

Administration of leptin to obese leptin-deficient rodents (e.g., *ob/ob* mice) and the few humans who have this condition

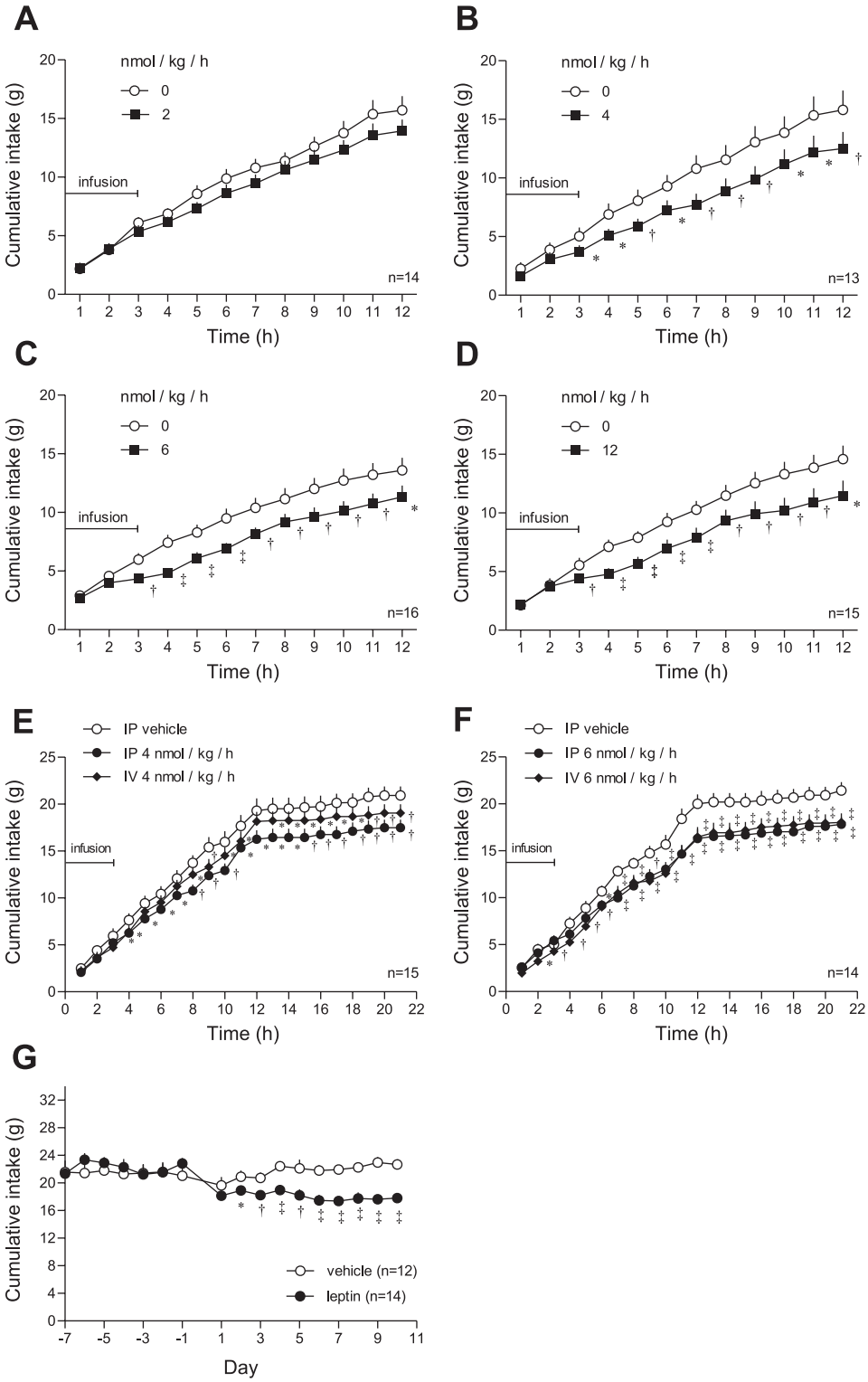

Fig. 3. *A–D*: effects of 3-h intravenous (iv) infusion of leptin on cumulative food intake during 12-h dark period in 13–16 lean rats. In a series of 4 experiments, normally fed rats received a 3-h jugular vein infusion of vehicle or leptin (2, 4, 6, or 12 nmol·kg^{-1}·h^{-1}) beginning 15 min before dark onset (*time 0*). *E* and *F*: effects of 3-h iv or intraperitoneal (ip) infusion of vehicle or leptin (4 or 6 nmol·kg^{-1}·h^{-1}) at dark onset on cumulative food intake for 21 h in 14–15 lean rats. Dark period was from 0 to 12 h. *G*: effects of two 3-h iv infusions of vehicle or leptin (4 nmol·kg^{-1}·h^{-1}) at onset of light and dark periods each day for 10 days on daily food intake of normal chow diet in lean rats. Values are means ± SE. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ compared with the vehicle-treated group.

reverses hypometabolism and hyperphagia and induces dramatic weight loss (47, 48). In contrast, most obese humans and DIO rodents are hyperleptinemic and leptin insensitive (24). Rosenbaum et al. (37) proposed that weight loss in obese subjects produces a relative leptin deficiency that activates orexigenic and energy conservation mechanisms in the brain to restore energy reserves and that leptin replacement would attenuate weight regain. In support of this hypothesis, they showed that a 10% weight reduction in obese humans reduces energy expenditure, satiation, and perception of the amount of food eaten and that low-dose leptin replacement reverses these effects (36, 37).

Here, we assessed whether hyperphagia and weight regain following a food restriction-induced weight loss of 10% in DIO

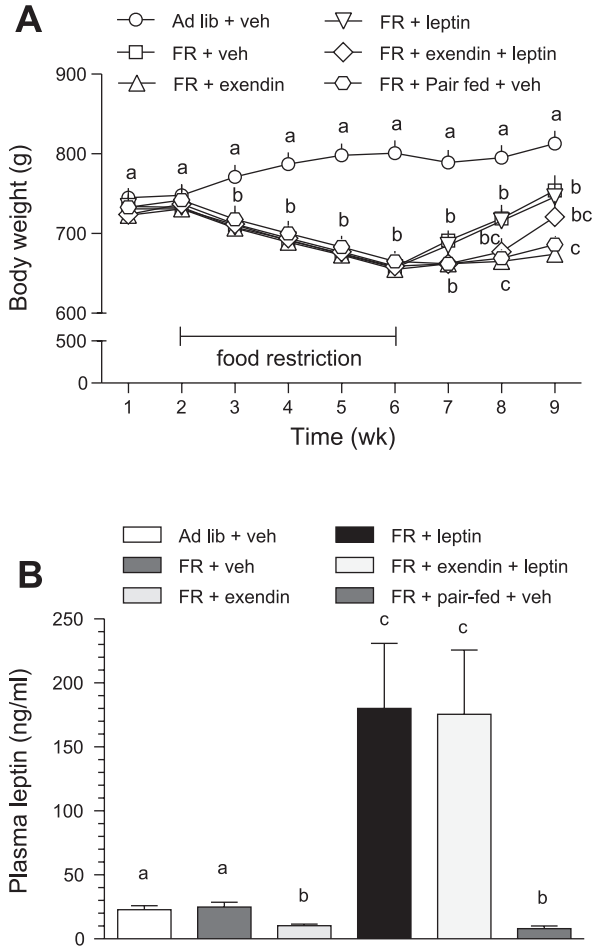

Fig. 4. Effects of 3-h ip infusions of leptin (4 nmol·kg^{-1}·h^{-1}), exendin-4 (50 pmol·kg^{-1}·h^{-1}), and leptin + exendin-4 at onset and end of dark period each day for 3 wk on body weight (*A*) and plasma leptin concentration (*B*) in DIO rats that had been 40% food restricted daily for 4 wk. Values are means ± SE. Values labeled with different letters are different ($P < 0.05$).

rats is mediated by a reduction in leptin signaling to the brain. Our results showed that weight loss in the DIO rats reversed hyperleptinemia, increased leptin sensitivity, and induced a postrestriction hyperphagia and weight (and fat) regain. However, daily systemic administration of leptin [two 3-h ip infusions at the onset and end of the dark period (24 nmol·kg^{-1}·day^{-1})] did not attenuate hyperphagia and weight regain, nor did it affect mean daily meal sizes or meal numbers in the weight-reduced DIO rats. Together, these results do not support the hypothesis that weight loss in obese subjects produces a relative leptin deficiency that activates orexigenic and energy conservation mechanisms in the brain to restore energy reserves.

Numerous studies have shown that acute systemic administration of long-acting GLP-1 receptor agonist exendin-4 can rapidly and potently suppress food intake in lean and obese subjects. Previously, we showed that in DIO rats two 3-h ip infusions of exendin-4 at the onset and the end of the dark period each day (0.15 nmol·kg^{-1}·day^{-1}) produced a sustained ~20% reduction in daily food intake for 17 days and decreased body weight by 7% (31). Here, we determined the effects of a similar daily regimen of exendin-4 dosing alone and with leptin (24 nmol·kg^{-1}·day^{-1}) for 3 wk on hyperphagia and weight regain in weight-reduced DIO rats. Exendin-4 alone prevented the post-restriction-induced increase in meal size, hyperphagia, and weight regain. Thus, intermittent ip infusion of exendin-4 had a similar prolonged effect on food intake whether administered to weight-reduced or non-weight-reduced DIO rats.

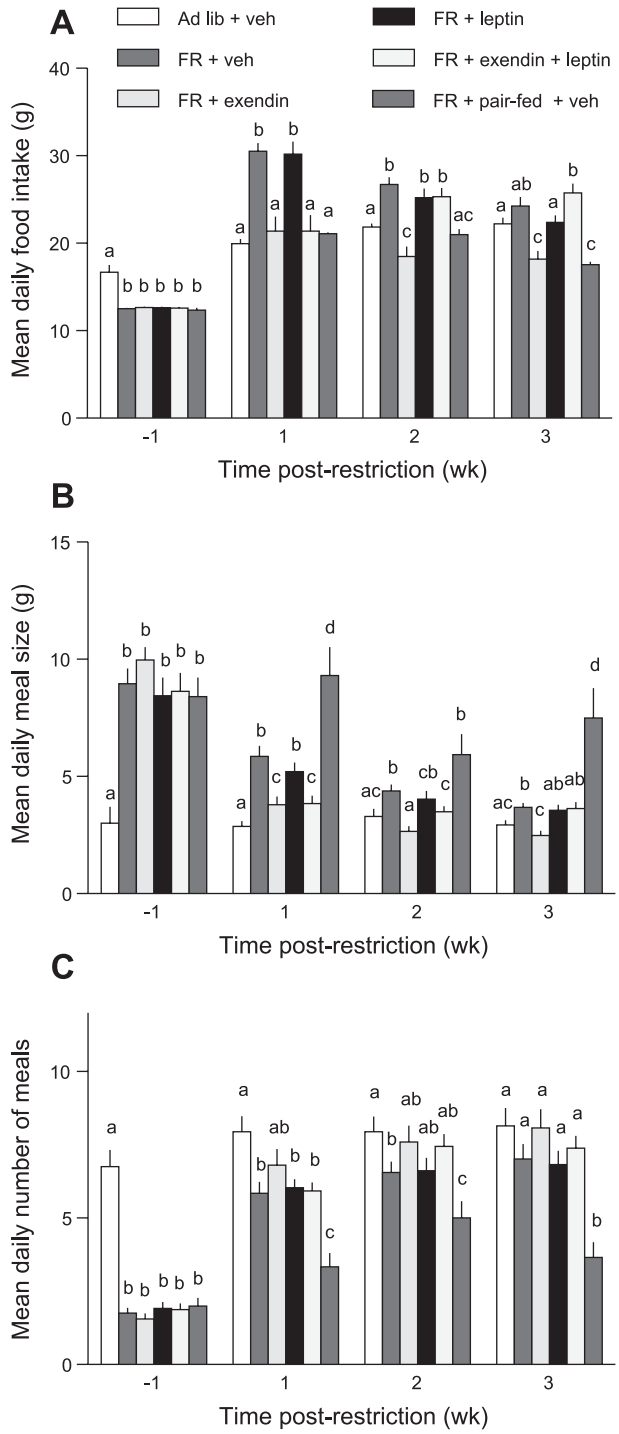

Fig. 5. Effects of 3-h ip infusions of leptin (4 nmol·kg^{-1}·h^{-1}), exendin-4 (50 pmol·kg^{-1}·h^{-1}), and leptin + exendin-4 at onset and end of dark period each day for 3 wk on mean daily food intake (*A*), mean daily meal size (*B*), and mean daily number of meals (*C*) in DIO rats that had been 40% food restricted daily for 4 wk. Values are means ± SE. Values labeled with different letters within each weekly period are different ($P < 0.05$).

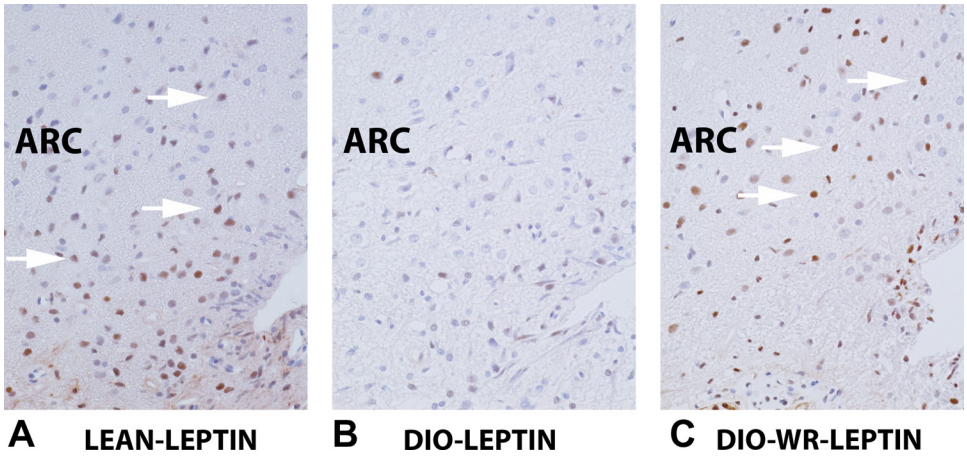

Fig. 6. Effects of ip injection of leptin (125 nmol/kg) on arcuate nucleus (ARC) phosphorylated signal transducer and activator of transcription 3 signaling (denoted by arrows) in age-matched lean rats (*A*), DIO rats (*B*), and 10% weight-reduced DIO rats (*C*) (×40 magnification).

Prevention of weight gain in the weight-reduced rats in response to exendin-4 administration was identical in rats pair-fed the daily amount of food ingested by the exendin-4-treated rats, which suggests that exendin-4 prevented weight regain by preventing hyperphagia. However, coadministration of leptin and exendin-4 did not reduce body weight more than exendin-4 alone. Rather, leptin began to attenuate the inhibitory effects of exendin-4 on hyperphagia and weight regain by the end of the 2nd wk of administration.

The inability of leptin replacement to reduce food intake and body weight in the weight-reduced DIO rats was not likely due to leptin insensitivity at the start of leptin administration, because age-matched lean and weight-reduced DIO rats exhibited comparable leptin sensitivities that were significantly greater than that observed in DIO rats. The inability of leptin replacement to reduce food intake and body weight in the weight-reduced DIO rats was also not likely due to leptin dosing being too low to affect food intake, because similar daily dosing produced a prolonged reduction in daily food intake and body weight in lean rats consuming normal rat chow (low fat). However, the weight-reduced DIO rats were provided a palatable high-fat diet instead of rat chow, and leptin replacement has been reported to be less effective in reducing intake of palatable food. Knight et al. (18) showed that a leptin replacement dose that prevented hyperphagia and weight gain in leptin-deficient *ob/ob* mice on a low-fat diet did not prevent development of obesity in *ob/ob* mice on a high-fat diet despite both groups of mice exhibiting normal leptin sensitivity. Furthermore, wild-type mice developed the same degree of obesity on the high-fat diet, yet they were hyperleptinemic and leptin insensitive. These results suggest that sustained hyperleptinemia, rather than chronic ingestion of a high-fat diet, produces leptin insensitivity and that leptin replacement alone may be unable to prevent weight regain in weight-reduced obese subjects exposed to palatable foods.

Another possible reason why leptin administration was unable to attenuate hyperphagia and weight regain in the weight-reduced DIO rats is that our dosing regimen may have rapidly produced leptin insensitivity. Our leptin-dosing regimen produced hyperleptinemia in the weight-reduced DIO rats, and hyperleptinemia can produce leptin insensitivity (18). In our study, plasma leptin levels in weight-reduced DIO rats receiving leptin infusions were sevenfold greater than those in weight-reduced DIO rats receiving vehicle infusions and 17-fold greater than those in weight-reduced DIO rats receiving exendin-4 infusions. Our leptin-dosing regimen also appeared to attenuate rather than enhance the inhibitory effect of exendin-4 on food intake and weight regain in the weight-reduced DIO rats. This would be consistent with the idea that the high leptin dose, by inducing leptin insensitivity, further reduced leptin signaling to the brain, which further activated orexigenic and energy conservation mechanisms to promote weight regain. Thus, it remains to be determined whether a leptin-dosing regimen that does not produce leptin insensitivity would attenuate hyperphagia and weight regain or enhance the efficacy of exendin-4 to reduce food intake and body weight in weight-reduced DIO rats.

No previous study has determined that chronic administration of leptin can attenuate weight regain or promote greater weight loss in weight-reduced obese individuals. Neither continuous SQ infusion nor twice daily ip injection of leptin prevented weight regain in weight-reduced DIO rats on a high-fat diet (5, 6). However, only single leptin doses were tested, and it is unclear whether they were ineffective because they produced hyperleptinemia and leptin insensitivity or because increased leptin signaling alone is unable to suppress eating of highly palatable foods (18, 24).

It remains to be determined whether chronic leptin administration can increase the efficacy of other anorexigenic substances to produce significant weight loss in obese subjects. Continuous SQ administration of leptin with either sibutramine (3) or amylin (40, 41) has been reported to synergistically reduce food intake and body weight in DIO rats. However, rats were likely not obese in these studies (i.e., relatively low %body fat and/or plasma leptin), and very little if any actual weight loss was produced by treatments: only 5% weight loss in the study by Boozer et al. (3) and only prevention of weight gain in the studies by Trevaskis and colleagues (40, 41). It is also unclear whether the continuous SQ doses of leptin used in these studies produced hyperleptinemia and leptin insensitivity.

In their recent review, Trevaskis et al. (42) reported that coadministration of leptin and amylin is much less effective in reducing body weight in heavier DIO-prone rats (550–800 g) than in the lighter DIO-prone rats (450 g) that they typically use and is not effective in humans with BMI >35. They suggested that this is due to greater leptin insensitivity in the more obese subjects. However, the apparent lower efficacy of

leptin and amylin to reduce body weight in heavier rats may be an artifact of the way they define weight loss as percent vehicle-corrected weight loss. A growing 450-g rat on a high-fat diet would be expected to gain weight more rapidly as a percentage of body weight than an older 800-g rat on the same diet. Thus, for an amylin-leptin treatment to produce the same percent vehicle-corrected weight loss in 450- and 800-g rats, a treatment that only prevents weight gain in 450-g rats would have to significantly reduce body weight in 800-g rats. Since reducing body weight likely activates counteracting orexigenic and energy conservation mechanisms much more so than does prevention of weight gain, then the apparent lower efficacy of amylin-leptin treatment in 800- vs. 450-g rats may be due to greater activation of counteracting mechanisms in the heavier rats rather than to greater leptin insensitivity. Thus, it remains to be determined whether coadministration of leptin and amylin or leptin with other anorexigenic substances can produce significant weight loss in DIO rats if weight loss is defined as percent change from initial body weight, as it is in human clinical trials. Furthermore, since chronic hyperleptinemia can cause leptin insensitivity, defining a leptin-dosing regimen that produces maximal leptin signaling to the brain is critical for determining whether leptin adjunct therapies can be effective.

ACKNOWLEDGMENTS

We thank John Behrendt, James Buescher, Danielle Gates, Tristan Gilmore, Dean Heimann, Brian Johnson, Linda Kelsey, Isamu Kishi, Savannah Light, Joyce Murphy, Rani Padanilam, Mary Randall, Keely Reidelberger, Kevin Reidelberger, Amanda Ross, Megan Ruland, Raquel Telfer, and Lucille Waite for their excellent technical assistance.

GRANTS

This work was performed at the Veterans Affairs Nebraska-Western Iowa Health Care System and Veterans Affairs Puget Sound Health Care System and was supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Rehabilitation Research, and Development Service and Biomedical Laboratory Research and Development. This research was also supported in part by the National Institutes of Health (R01-DK-70851 and P20-RR-16469) and the Cellular and Molecular Imaging Core of the National Institutes of Health Diabetes Research Center (P30-DK-17047). The contents of this article do not represent the views of the Department of Veterans Affairs or the US Government.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.R., A.C.H., P.K.C., and J.E.B. did the conception and design of the research; R.R., A.C.H., P.K.C., B.A., C.P.-O., K.A., S.S., and J.E.B. performed the experiments; R.R., A.C.H., P.K.C., B.A., C.P.-O., K.A., S.S., and J.E.B. analyzed the data; R.R., A.C.H., P.K.C., B.A., C.P.-O., and J.E.B. interpreted the results of the experiments; R.R. and J.E.B. prepared the figures; R.R. and J.E.B. drafted the manuscript; R.R., A.C.H., P.K.C., B.A., and J.E.B. edited and revised the manuscript; R.R., A.C.H., P.K.C., and J.E.B. approved the final version of the manuscript.

REFERENCES

1. **Abbott CR, Small CJ, Sajedi A, Smith KL, Parkinson JR, Broadhead LL, Ghatei MA, Bloom SR.** The importance of acclimatisation and habituation to experimental conditions when investigating the anorectic effects of gastrointestinal hormones in the rat. *Int J Obes Relat Metab Disord* 30: 288–292, 2006.
2. **Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR.** Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature* 418: 650–654, 2002.
3. **Boozer CN, Leibel RL, Love RJ, Cha MC, Aronne LJ.** Synergy of sibutramine and low-dose leptin in treatment of diet-induced obesity in rats. *Metabolism* 50: 889–893, 2001.
4. **Bray GA, Tartaglia LA.** Medicinal strategies in the treatment of obesity. *Nature* 404: 672–677, 2000.
5. **Buison A, Ordiz F, Jen KL.** Long-term effects of exogenous leptin on body weight and fat in post-obese female rats. *Physiol Behav* 74: 321–328, 2001.
6. **Buison A, Pellizzon M, Ordiz F, Jen KL.** Augmenting leptin circadian rhythm following a weight reduction in diet-induced obese rats: short- and long-term effects. *Metabolism* 53: 782–789, 2004.
7. **Chelikani PK, Haver AC, Reeve JR Jr, Keire DA, Reidelberger RD.** Daily, intermittent intravenous infusion of peptide YY(3–36) reduces daily food intake and adiposity in rats. *Am J Physiol Regul Integr Comp Physiol* 290: R298–R305, 2006.
8. **Chelikani PK, Haver AC, Reidelberger RD.** Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* 288: R1695–R1706, 2005.
9. **Chelikani PK, Haver AC, Reidelberger RD.** Intravenous infusion of peptide YY(3–36) potently inhibits food intake in rats. *Endocrinology* 146: 879–888, 2005.
10. **Chelikani PK, Haver AC, Reidelberger RD.** Effects of intermittent intraperitoneal infusion of salmon calcitonin on food intake and adiposity in obese rats. *Am J Physiol Regul Integr Comp Physiol* 293: R1798–R1808, 2007.
11. **Chelikani PK, Haver AC, Reidelberger RD.** Intermittent intraperitoneal infusion of peptide YY(3–36) reduces daily food intake and adiposity in obese rats. *Am J Physiol Regul Integr Comp Physiol* 293: R39–R46, 2007.
12. **Faouzi M, Leshan R, Björnholm M, Hennessey T, Jones J, Münzberg H.** Differential accessibility of circulating leptin to individual hypothalamic sites. *Endocrinology* 148: 5414–5423, 2007.
13. **Finer N.** Pharmacotherapy of obesity. *Best Pract Res Clin Endocrinol Metab* 16: 717–742, 2002.
14. **Grady EF, Bohm SK, Bunnett NW.** Turning off the signal: mechanisms that attenuate signaling by G protein-coupled receptors. *Am J Physiol Gastrointest Liver Physiol* 273: G586–G601, 1997.
15. **Gura T.** Obesity research: new data on appetite-suppressing peptide challenge critics. *Science* 306: 1453–1454, 2004.
16. **Harris RB, Mitchell TD, Hebert S.** Leptin-induced changes in body composition in high fat-fed mice. *Exp Biol Med (Maywood)* 228: 24–32, 2003.
17. **Hübschle T, Thom E, Watson A, Roth J, Klaus S, Meyerhof W.** Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. *J Neurosci* 21: 2413–2424, 2001.
18. **Knight ZA, Hannan KS, Greenberg ML, Friedman JM.** Hyperleptinemia is required for the development of leptin resistance. *PLoS One* 5: e11376, 2010.
19. **Korner J, Inabnet W, Febres G, Conwell IM, McMahon DJ, Salas R, Taveras C, Schrope B, Bessler M.** Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass. *Int J Obes (Lond)* 33: 786–795, 2009.
20. **Le Roux CW, Welbourn R, Werling M, Osborne A, Kokkinos A, Laurenius A, Lonroth H, Fandriks L, Ghatei MA, Bloom SR, Olbers T.** Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg* 246: 780–785, 2007.
21. **Levin BE.** Factors promoting and ameliorating the development of obesity. *Physiol Behav* 86: 633–639, 2005.
22. **Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW.** Central nervous system control of food intake and body weight. *Nature* 443: 289–295, 2006.
23. **Münzberg H, Flier JS, Bjørbaek C.** Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145: 4880–4889, 2004.
24. **Myers MG, Leibel RL, Seeley RJ, Schwartz MW.** Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab* 21: 643–651, 2010.
25. **Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC, Novelli Filho JL.** Anthropometrical parameters and markers of obesity in rats. *Lab Anim* 41: 111–119, 2007.
26. **Paxinos G, Watson C.** *The Rat Brain in Stereotaxic* Coordinates (6th ed). Amsterdam: Academic, 2007.

27. **Persson P.** Highlights from the literature: What underlies the inability of several labs to reproduce the appetite suppressing effects of peptide YY(3–36) [PYY(3–36)]? *Physiology* 21: 4–5, 2006.
28. **Pittner RA, Moore CX, Bhavsar SP, Gedulin BR, Smith PA, Jodka CM, Parkes DG, Paterniti JR, Srivastava VP, Young AA.** Effects of PYY[3–36] in rodent models of diabetes and obesity. *Int J Obes Relat Metab Disord* 28: 963–971, 2004.
29. **Reidelberger R, Haver A, Apenteng B, Anders K.** Effects of intermittent intraperitoneal infusion of amylin on food intake, body weight and adiposity in diet-induced obese rats (Abstract). *Obesity (Silver Spring)* 17: S175, 2009.
30. **Reidelberger RD, Arnelo U, Granqvist L, Permert J.** Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* 280: R605–R611, 2001.
31. **Reidelberger RD, Haver AC, Apenteng BA, Anders KL, Steenson SM.** Effects of exendin-4 alone and with peptide YY(3–36) on food intake and body weight in diet-induced obese rats. *Obesity (Silver Spring)* 19: 121–127, 2011.
32. **Reidelberger RD, Haver AC, Chelikani PK, Buescher JL.** Effects of different intermittent peptide YY (3–36) dosing strategies on food intake, body weight, and adiposity in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 295: R449–R458, 2008.
33. **Reidelberger RD, Haver AC, Chelikani PK, Buescher JL, Chowdhury A.** Effects of intermittent intraperitoneal infusion of opiate antagonist naltrexone on food intake, body weight and adiposity in diet-induced obese rats (Abstract). *Obesity (Silver Spring)* 16: S108, 2008.
34. **Reidelberger RD, Kelsey L, Heimann D.** Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* 282: R1395–R1404, 2002.
35. **Robertson SA, Leinninger GM, Myers MG.** Molecular and neural mediators of leptin action. *Physiol Behav* 94: 637–642, 2008.
36. **Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, Leibel RL.** Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest* 115: 3579–3586, 2005.
37. **Rosenbaum M, Kissileff HR, Mayer LE, Hirsch J, Leibel RL.** Energy intake in weight-reduced humans. *Brain Res* 1350: 95–102, 2010.
38. **Roth JD, Hughes H, Kendall E, Baron AD, Anderson CM.** Antiobesity effects of the beta-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. *Endocrinology* 147: 5855–5864, 2006.
39. **Salvà Lacombe P, García Vicente JA, Costa Pagès J, Lucio Morselli P.** Causes and problems of nonresponse or poor response to drugs. *Drugs* 51: 552–570, 1996.
40. **Trevaskis JL, Coffey T, Cole R, Lei C, Wittmer C, Walsh B, Weyer C, Koda J, Baron AD, Parkes DG, Roth JD.** Amylin-mediated restoration of leptin responsiveness in diet-induced obesity: magnitude and mechanisms. *Endocrinology* 149: 5679–5687, 2008.
41. **Trevaskis JL, Lei C, Koda JE, Weyer C, Parkes DG, Roth JD.** Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats. *Obesity (Silver Spring)* 18: 21–26, 2010.
42. **Trevaskis JL, Parkes DG, Roth JD.** Insights into amylin-leptin synergy. *Trends Endocrinol Metab* 21: 473–479, 2010.
43. **Tschöp M, Castañeda TR, Joost HG, Thöne-Reineke C, Ortmann S, Klaus S, Hagan MM, Chandler PC, Oswald KD, Benoit SC, Seeley RJ, Kinzig KP, Moran TH, Beck-sickinger AG, Koglin N, Rodgers RJ, Blundell JE, Ishii Y, Beattie AH, Holch P, Allison DB, Raun K, Madsen K, Wulff BS, Stidsen CE, Birringer M, Kreuzer OJ, Schindler M, Arndt K, Rudolf K, Mark M, Deng XY, Whitcomb DC, Halem H, Taylor J, Dong J, Datta R, Culler M, Craney S, Flora D, Smiley D, Heiman ML.** Physiology: does gut hormone PYY3–36 decrease food intake in rodents? *Nature* 430: 165, 2004.
44. **Vrang N, Madsen AN, Tang-Christensen M, Hansen G, Larsen PJ.** PYY(3–36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 291: R367–R375, 2006.
45. **Weeks JR, Myers RD.** Long-term intravenous infusion. In: *Methods in Psychobiology*. London: Academic, 1972.
46. **Williams DL, Baskin DG, Schwartz MW.** Hindbrain leptin receptor stimulation enhances the anorexic response to cholecystokinin. *Am J Physiol Regul Integr Comp Physiol* 297: R1238–R1246, 2009.
47. **Williamson DA, Ravussin E, Wong ML, Wagner A, Dipaoli A, Caglayan S, Ozata M, Martin C, Walden H, Arnett C, Licinio J.** Microanalysis of eating behavior of three leptin deficient adults treated with leptin therapy. *Appetite* 45: 75–80, 2005.
48. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425–432, 1994.
49. **Zorrilla EP, Inoue K, Fekete EM, Tabarin A, Valdez GR, Koob GF.** Measuring meals: structure of prandial food and water intake of rats. *Am J Physiol Regul Integr Comp Physiol* 288: R1450–R1467, 2005.