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Leptin Responsiveness in Chronically Decerebrate Rats

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Peripheral infusions of physiological doses of leptin decrease body fat mass, but it is not known whether this results from direct effects on peripheral tissue or activation of central leptin receptors. In this study, we infused chronically decerebrate (CD) rats, in which the forebrain was surgically isolated from the caudal brainstem, with 60 μg leptin/d or PBS for 14 d from ip mini-osmotic pumps. The CD rats were tube fed an amount of food equivalent to the intake of *ad libitum*-fed intact controls or 75% of this amount to account for their reduced energy expenditure. Control rats fed *ad libitum* or tube fed 75, 100, or 125% of their *ad libitum* intake also were peripherally infused with leptin or PBS. CD rats had a lower serum testosterone, energy expenditure, and lean body mass compared with controls but had increased levels of adiponectin and leptin and were obese. Leptin increased body fat and decreased energy expenditure during the light period in 100%-fed CD rats, but not 75%-fed CD rats. Leptin decreased body fat of *ad libitum*- and 100%-fed but not 75%-fed or 125%-fed intact controls. Energy expenditure did not change in any control group. These results show that leptin can change body fat independent of a change in food intake or energy expenditure, that the forebrain normally prevents leptin from inhibiting energy expenditure through mechanisms initiated in the caudal brainstem or peripheral tissues, and that the leptin response in both intact and CD rats is determined by the energy status of the animal. (*Endocrinology* 148: 4623–4633, 2007)

LEPTIN, A HORMONE PRODUCED predominantly by adipose tissue, was initially hypothesized to function as a circulating feedback signal in the regulation of energy balance (1). Subsequent investigations have shown that leptin has multiple physiological activities, and its primary function remains a subject of discussion (2). It is clear, however, that the absence of leptin results in severe obesity in both rodents (3) and humans (4) and that administration of leptin can inhibit food intake, increase energy expenditure, and cause a selective loss of body fat in both leptin-sufficient and leptin-deplete animals (5–7). The magnitude of each of these responses is determined by the dose of leptin and whether it is administered centrally or peripherally. In previous studies, we have used a rodent model in which low doses of leptin are infused peripherally from mini-osmotic pumps to produce physiological elevations in circulating leptin in mice or rats (5, 8, 9). Over a 2-wk infusion period, this treatment results in a selective loss of body fat without necessarily producing any significant inhibition of food intake. The strength of this model is that it is more closely representative of the normal physiological release of leptin than are bolus injections of leptin into either the periphery or the cerebral ventricles.

The leptin-induced change in body composition, which is characterized by a loss of fat but retention of lean body mass (5), could result from leptin inducing a state of negative energy balance by inhibiting energy intake, increasing energy expenditure, or both. Energy would then be mobilized from body energy stores to support metabolism. The changes in energy intake or expenditure required to produce a significant loss of body fat over a period of 2 wk could potentially be very small and difficult to detect with 24-h measures. Alternatively, leptin could cause a change in nutrient partitioning without disrupting energy balance, such that a shift in metabolism favors lean body mass but inhibits deposition of lipid in adipose tissue. It is established that the inhibitory effect of leptin on food intake is mediated by leptin receptors in neural tissue (10) and that leptin-induced changes in energy expenditure are mediated by increased activity of uncoupling protein 1 (UCP1) in brown adipose tissue (11, 12), which also appears to require activation of leptin receptors in the forebrain, midbrain, or caudal brainstem (13, 14). In contrast, if the change in body composition is due to a shift in the metabolic equilibrium in peripheral tissues, then it is possible that leptin acts directly on adipose and liver tissue to inhibit lipogenesis and mobilize lipid independent of activation of leptin receptors in the brain. Most peripheral tissues express one or more subtypes of the leptin receptor (15), although the subtypes with short intracellular domains dominate. Expression of ObRb, the leptin receptor subtype that has a long-intracellular domain, is relatively very low in peripheral tissues of rodents with the lung being an exception (16). Evidence of leptin activity in db/db mice that do not express ObRb (17) and a recent report that leptin can act directly to mobilize liver lipid (18) demonstrates that the leptin receptor isoforms with short-intracellular domains have the potential to mediate some metabolic responses to leptin because ObRb mRNA and protein is undetectable in hepatic tissue (19).

To determine whether changes in body composition of rats receiving peripheral, low-dose infusions of leptin require

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Abbreviations: AL, *Ad libitum*; CD, chronically decerebrate; RER, respiratory equivalency ratio; UCP1, uncoupling protein 1.

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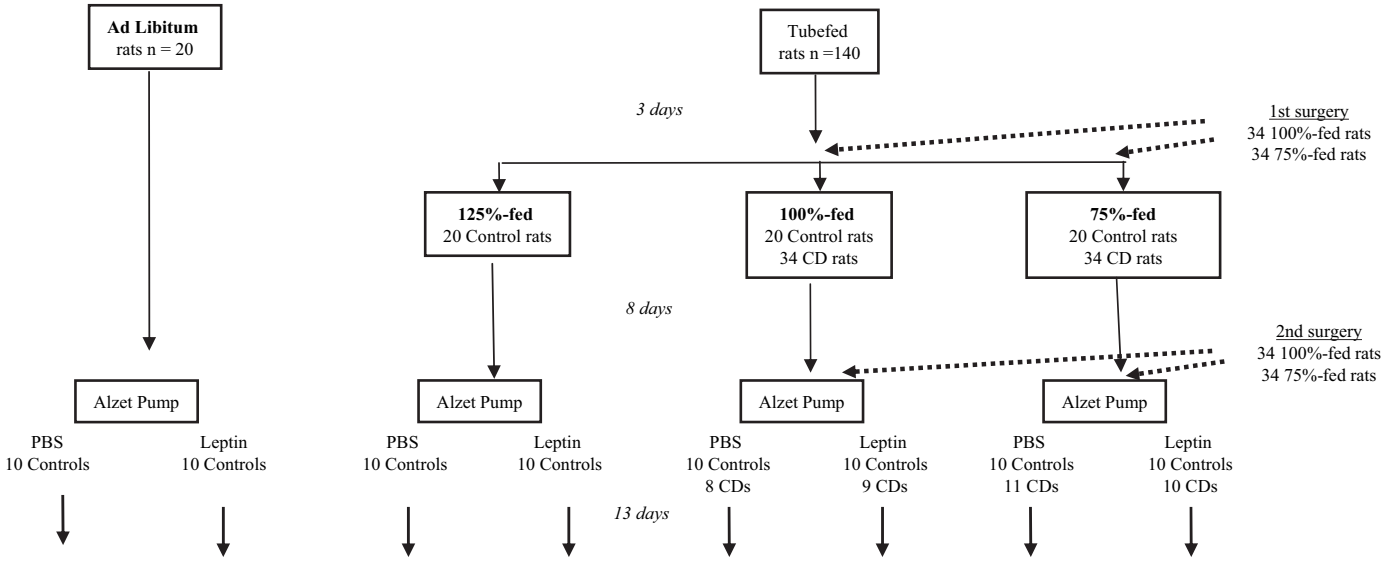

FIG. 1. Experimental design.

leptin to cross the blood-brain barrier and activate hypothalamic leptin receptors, we used the chronically decerebrate (CD) rat. In this model, a surgical transection is made to isolate the caudal brainstem from the forebrain, and it has been used extensively to demonstrate that many feeding and energetic responses are intact in the absence of neural input from the forebrain (20, 21). The caudal brainstem contains receptors for many of the neuropeptides and receptors that are known to be important in the control of food intake (20) including those for leptin (22, 23), and it also is an integral part of the sympathetic neural circuit controlling peripheral tissues (14, 24–26). Although CD rats do not eat or drink spontaneously, studies in which food is infused directly into the oral cavity (intra-oral feeding) have shown that they exhibit normal short-term regulation of meal size (see Ref. 20 for review). Most recently, we demonstrated that CD rats made appropriate changes in energy expenditure and lipid mobilization in response to 48 h of starvation (27) and also show an increase in mRNA expression of UCP1 in intrascapular brown adipose tissue (IBAT) after fourth ventricle infusion of the melanocortin receptor agonist melanotan II (28).

The objective of this study was to determine whether CD rats receiving a peripheral infusion of leptin would show the same changes in body composition as those found in neurally intact control rats. The absence of neural involvement of the forebrain would allow us to determine the requirement for activation of the hypothalamic leptin receptors in the mediation of changes in body composition produced by peripheral infusions of leptin.

Materials and Methods

All experimental procedures described here were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 148 male Sprague Dawley rats (275–300 g: Harlan, Indianapolis, IN), in two cohorts, were housed in individual cages in a room maintained at 23 C with lights on for 12 h each day from 0600 h. All of the rats had free access to rodent chow (Rodent Chow 5001; Purina Mills, St. Louis, MO) and water for 1 wk of adaptation. Thirty of the rats were selected at random, housed in hanging wire-mesh cages, and offered suspendible AIN 76A rodent diet (L1001; Research Diets, New Brunswick, NJ) in dry form so that voluntary food intake, corrected for spillage, could be recorded. Daily body weights of all of the rats and food intakes of the 30 rats were recorded for 1 wk. At the end of this week, all of the rats in the experiment were switched to the suspendible diet and were divided into two groups: 20 rats fed the dry diet *ad libitum* (AL-fed) and 140 rats tube fed the diet in liquid form in three meals each day fed at 0600, 1400, and 2200 h. The volume of tube-fed meals was gradually increased from 9 to 12 ml over 2 d to deliver a total of 79 kcal/d, which represented 85% of the voluntary food intake of the AL-fed rats. This level of feeding has previously been shown to produce a similar rate of weight gain as that of AL-fed rats (29) and in this study will be referred to as 100%-fed. The liquid diet also provided an adequate daily water intake for the animals, even though water bottles were always available. The 20 AL-fed rats were selected as the rats with the median body weight of all of the rats. After 3 d of tube feeding, the tube-fed animals were divided into three weight-matched groups: 100%-fed (54 rats), 75%-fed (54 rats), and 125%-fed (20 rats). Thirty-four 100%-fed rats and 34 75%-fed rats were subjected to the first stage of surgery for chronic decerebration (CD) as described previously (27). The 100%-fed rats continued to receive 79 kcal/d, intake of the 75%-fed rats was reduced to 59 kcal/d, and intake of the 125%-fed rats was increased over 3 d to 99 kcal/d. Eight days after the first surgery, the CD rats were subjected to a second surgery to produce a complete sectioning of the neuraxis made at the mesencephalic-diencephalic juncture (27). At the same time as the second surgery, all rats (CD plus intact controls) were fitted with an ip Alzet mini-osmotic pump (model 2002; Diurect Corp., Cupertino, CA) that delivered either PBS or 60 μg leptin/d (rat recombinant leptin; R&D Systems, Minneapolis, MN). A total

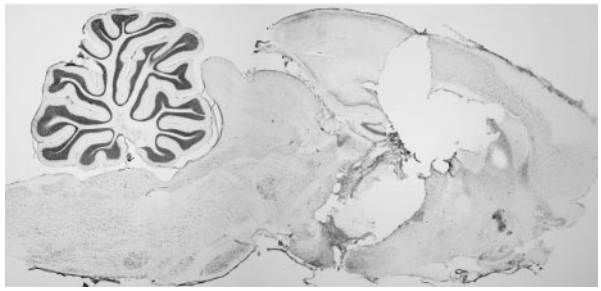

FIG. 2. A typical midline sagittal section from a CD rat demonstrating the efficacy of the lesion.

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of 38 of the 68 CD rats survived both surgeries (10 leptin-infused 75%-fed, 11 PBS-infused 75%-fed, nine leptin-infused 100%-fed, and eight PBS-infused 100%-fed). The experimental design is summarized in Fig. 1.

Daily body weights of all rats were recorded each morning before the first tube-fed meal, and daily food intakes were recorded for the AL-fed rats. Rectal temperatures of the CD rats were measured using a thermistor probe (Temp 4, Thermistor Thermometer; Cole Parmer Instrument Co., Vernon Hills, IL) before each tube-fed meal. On the day after complete decerebration, rats were warmed with a heating pad if rectal temperature fell below 34 C. By the second day after surgery, all of the CD rats were maintaining their temperatures above 34 C, and we stopped measuring rectal temperatures the next day. Three days after the second surgery, the third day of leptin infusion, a tail-blood sample was collected from each rat to measure serum leptin concentrations (rat leptin RIA kit; Linco Research Inc., St. Charles, MO). Between d 4 and 11 of the leptin infusion, the energy expenditure of each rat was measured for a 26-h period using an indirect calorimeter, as described previously (27). Because the calorimeter could house only 16 animals at a time, rats from each treatment group were represented as evenly as possible within each set that went into the calorimeter. The rats were housed in the calorimeter for approximately 20 h before any experimental data were collected, and they were maintained on their normal feeding schedule. Heat production and respiratory equivalency ratio (RER) was measured on each cage every 20 min from 1100 h on one day until 1300 h on the following day. The rats were then returned to their home cages.

On the 13th day of leptin infusion, all of the tube-fed rats received a morning meal of 13.1 kcal (6 ml) at 0600 h. Food was removed from the cages of the AL-fed rats. Starting from 1000 h, the rats were killed by decapitation. Trunk blood was collected for measurement of serum glucose, insulin (rat insulin RIA kit; Linco Research), adiponectin (mouse adiponectin RIA; Linco Research), free fatty acids (NEFA C kit; Wako Chemical Co., Dallas, TX), triglycerides (kit from Sigma Chemical Co., St. Louis, MO), glycerol (Sigma), testosterone (total testosterone RIA; Diagnostic Systems Laboratories, Webster, TX), TSH (Diagnostic Systems), and corticosterone (rat corticosterone RIA; MP Biomedicals,

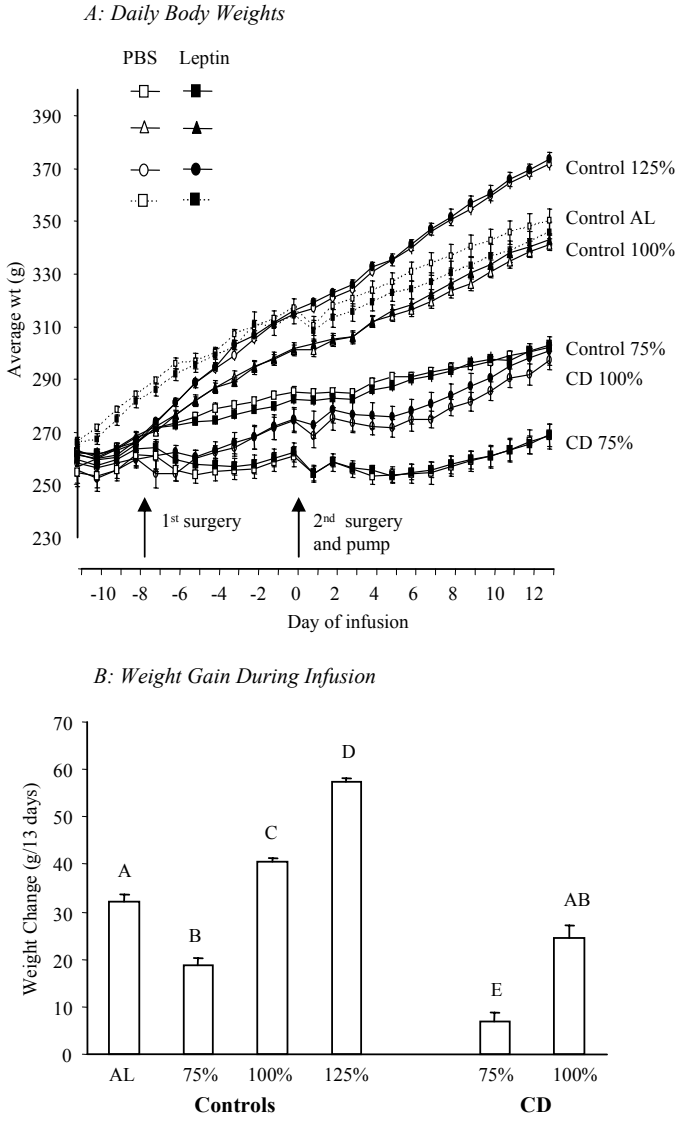

FIG. 3. Daily body weight (A) and weight gain during the period of PBS or leptin infusion (d 0–13) (B). Data are means + SEM for groups of eight to 11 rats in A and for 17–21 rats in B. Values in B that do not share a common *superscript* are significantly different at $P < 0.05$. Leptin did not have a significant effect on body weight or weight gain of any treatment group.

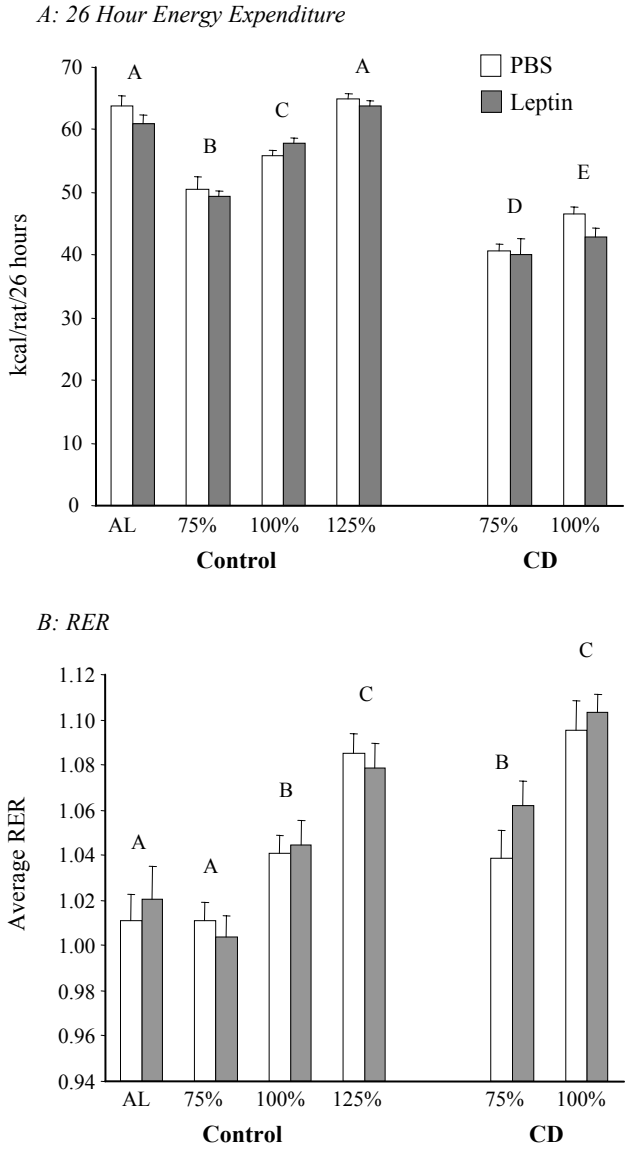

FIG. 4. Twenty-six-hour heat production (A) and average RER (B). Cohorts of 16 rats were placed in the calorimeter for 47 h between 4 and 11 d of infusion, and data were collected for the last 26 h. Data are means + SEM for groups of eight to 11 rats. *Superscript letters* indicate significant differences between treatment groups ($P < 0.05$).

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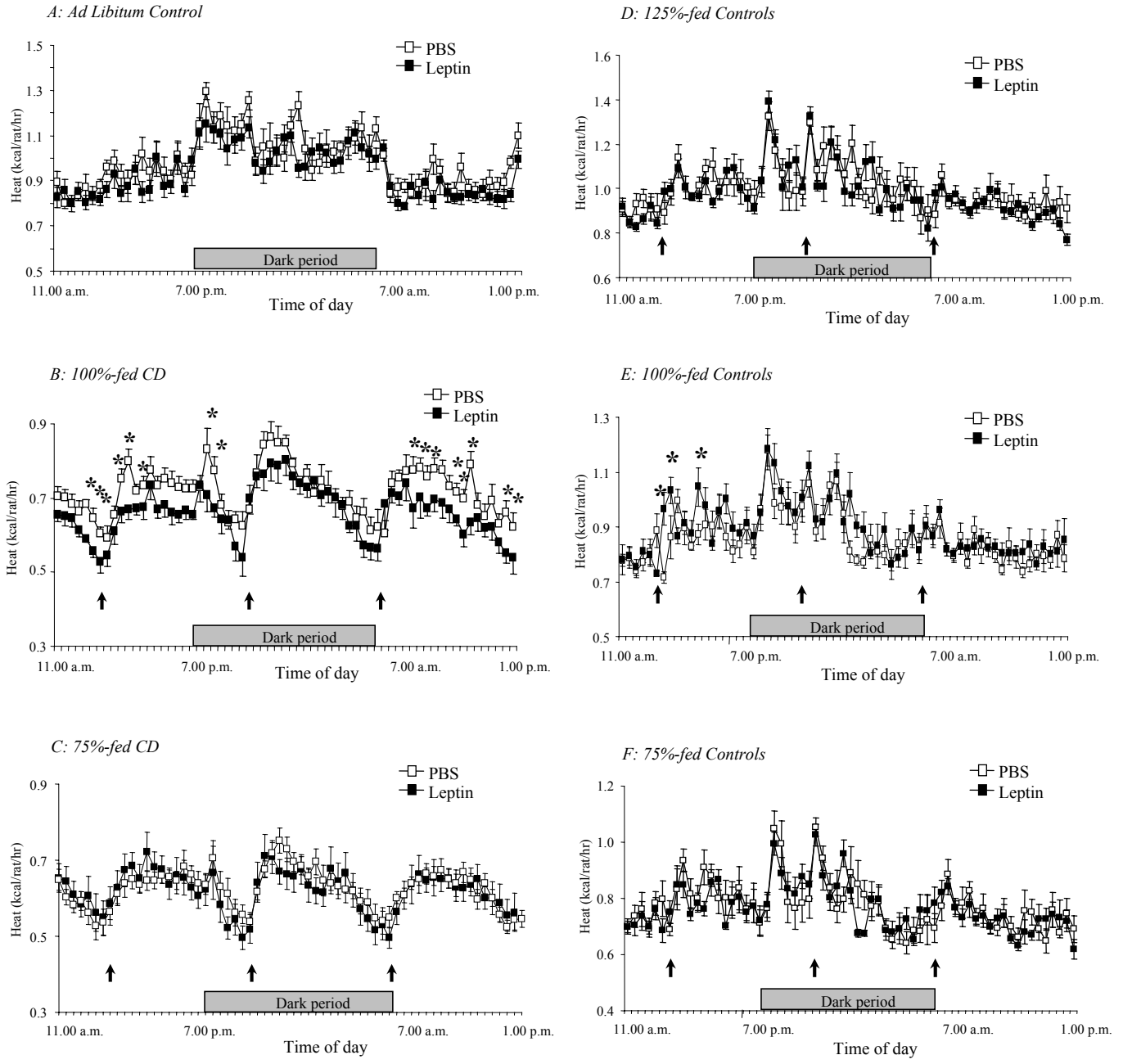

FIG. 5. Heat production measured over 26 h during the period of leptin or PBS infusion for each treatment group. Data are means ± SEM for groups of eight to 11 rats. An *asterisk* indicates a 25-min time interval during which there was a significant ($P < 0.05$) effect of leptin on energy expenditure.

Cost Mesa, CA). The left epididymal, inguinal, retroperitoneal, and perirenal white fat and the mesenteric fat pad were dissected and weighed. Small pieces of epididymal and retroperitoneal fat were fixed in osmium tetroxide for determination of fat cell size and number, as described previously (30). Liver, IBAT, testes, and adrenal glands were also dissected and weighed. Brains of CD rats were examined histologically to confirm the completeness of the transection (see Fig. 2). One lobe of the liver was frozen for measurement of liver lipid and glycogen content as described previously (31). The adrenal glands were snap frozen for determination of tissue norepinephrine and epinephrine content by HPLC, as described previously (9). Remaining tissues were returned to the carcass. The gastrointestinal tract was removed and carcass composition determined (32).

Statistical analysis

Because of the unbalanced nature of the experimental design, data were analyzed in three steps. Initially, a one-way ANOVA was conducted make an overall comparison of intact control and CD rats. Subsequently, two-way ANOVAs were conducted to test for feeding level and leptin infusion within either the control or CD groups. If there was no significant effect of leptin in any of the treatment groups, then the values for PBS and leptin-infused rats were combined and the effects of feeding level alone determined. *Post hoc* Duncan's multiple range test was used to determine differences between specific groups with $P < 0.05$ considered significantly different. Superscript letters are used to indicate significant differences between treatment groups, and asterisks indicate

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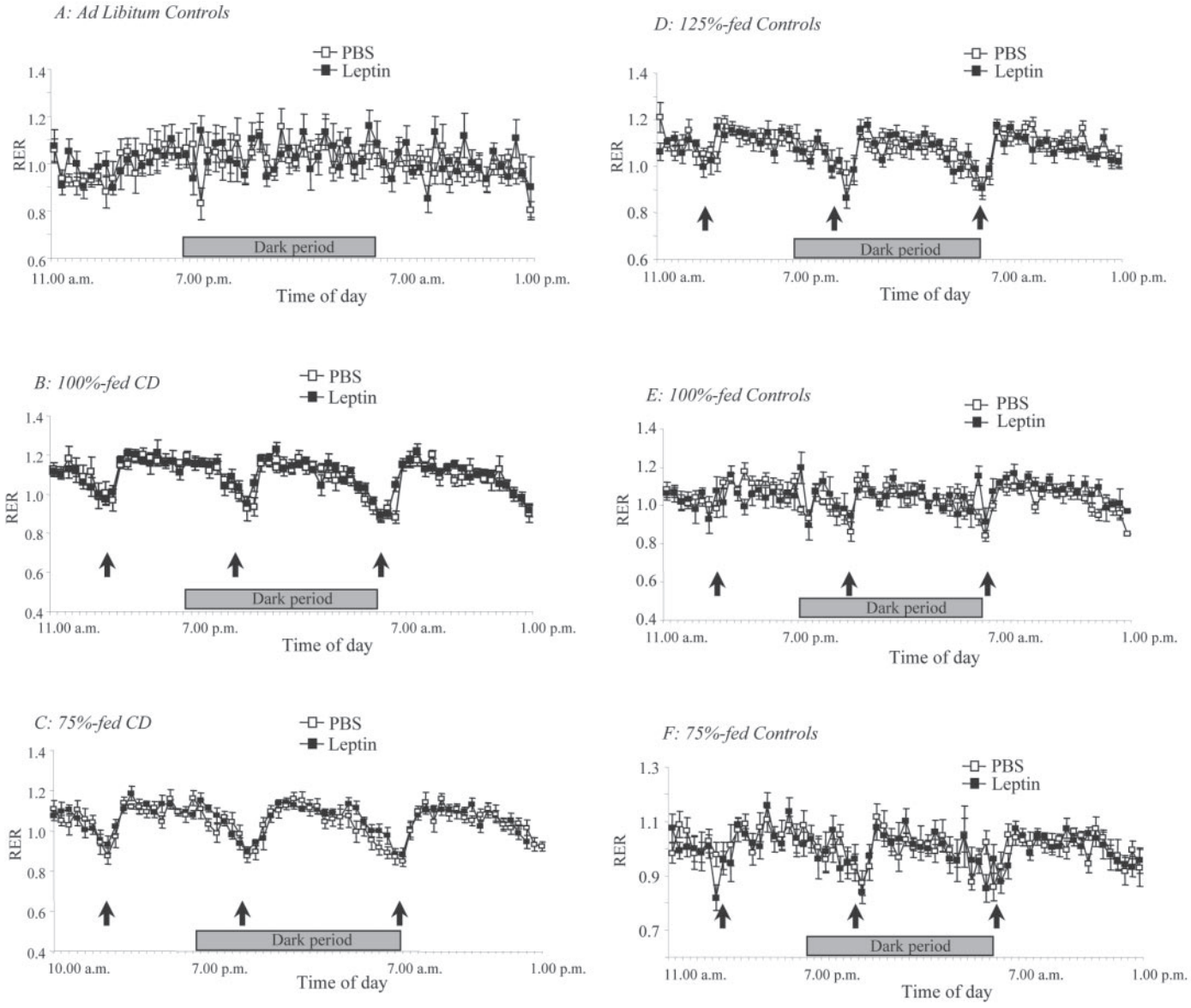

FIG. 6. RER during the 26 h that heat production was measured for each treatment group. Data are means + SEM for groups of eight to 11 rats. There was no significant effect of leptin on RER in any group at any time point.

a significant effect of leptin within that group. All statistical analysis was performed using Statistica Software (StatSoft, Tulsa, OK).

Results

Rats subjected to the CD surgery stopped gaining weight after the unilateral transection (see Fig. 3A) and gained less weight during the experimental period, after complete transection, than control rats fed an equivalent amount of food (Fig. 3B). The 75%-fed control rats gained less weight than AL-fed controls, whereas the 100%-fed and 125%-fed controls gained significantly more weight than AL-fed rats (Fig. 3B). There was no effect of leptin infusion on the food intakes of AL-fed rats (PBS-infused = 247 ± 7 g/12 d; leptin-infused = 244 ± 7 g/12 d). In a previous study, when all meals were fed within 12 h, body temperature of CD rats showed significant excursions in the days immediately after the second surgery (27). In the study described here, meals were spread evenly across 24 h, and rectal temperatures were stable at approximately 35.5 C within the first 3 d after the second surgery.

Total 26-h energy expenditure during the experimental period was significantly lower in CD than control rats, and the 75%-fed CD rats had a lower expenditure than 100%-fed CD rats (Fig. 4A). The 26-h expenditure of AL-fed controls was higher than in 75%-fed or 100%-fed control rats but the same as that of 125%-fed controls (Fig. 4A). Leptin caused a suggestive but nonsignificant reduction in 26-h expenditure of 100%-fed CD rats (PBS = 47 ± 1 kcal/26 h; leptin = 43 ± 1 kcal/26 h; $P < 0.06$ by two-tailed t test). This was associated with a significant reduction in expenditure during the light period (leptin, $P < 0.002$; interval, $P < 0.0001$; interaction, not significant) but not the dark period (Fig. 5B). In 100%-fed

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controls, there was no simple effect of leptin on expenditure in the light period (Fig. 5E: leptin, not significant; interval, $P < 0.001$; interaction, $P < 0.01$) and no effect during the dark period. In contrast, in the 75%-fed CD rats, there was no effect of leptin on expenditure during the light period but an interaction between leptin and time interval during the dark period (Fig. 5C: leptin, not significant; interval, $P < 0.001$; interaction, $P < 0.05$), even though there was no specific time point at which there was a significant difference between the PBS- and leptin-infused rats. There was no effect of leptin on expenditure during the light or dark period in any other group of rats (Fig. 5).

Average RER measured over 26 h was higher in CD than control rats fed the same amount of food (Fig. 4B) and averaged above 1.0, implying that even the 75%-fed CD rats were depositing energy into body stores (33). In intact control rats, the RER of AL-fed and 75%-fed rats was approximately 1.0, suggesting that the rats were predominantly oxidizing carbohydrate for energy (33), whereas the RER for both 100%-fed and 125%-fed rats was above 1.0, again suggesting that these rats were storing energy. As described in *Materials and Methods*, the 100%-fed rats were actually tube fed 85% of AL intake due to the increased efficiency of energy utilization in meal-fed rats (34), but even at this level of feeding, the 100%-fed rats had a higher RER than AL-fed rats. Leptin had no effect on RER for any treatment group. When RER was plotted by time interval (Fig. 6), it was more stable in intact control rats than in CD rats. Both groups of CD rats showed dips in RER toward 0.8 just before each meal, indicating that the rats were oxidizing fat and protein (33), and similar changes in RER were apparent in 75%-fed but not 100%-fed control rats.

At the end of the 13-d experimental period, both 75%-fed and 100%-fed CD rats were significantly fatter than control rats fed the same food intake (Fig. 7A). Reducing the food intake of the 75%-fed CD rats to match their energy expenditure did not prevent the obesity observed in the 100%-fed CD rats but inhibited weight gain even more than in 100%-fed CD rats (feeding level, $P < 0.0001$; leptin, $P < 0.03$; interaction, not significant). Leptin caused a significant increase in carcass fat content of 100%-fed CD rats that had similar amounts of carcass fat (grams per rat) as 125%-fed control rats, even though their carcasses were 20% smaller (Table 1). In intact control rats, body fat was the same in AL-fed and 100%-fed groups, reduced in 75%-fed rats, and increased in 125%-fed rats (feeding level, $P < 0.0001$; leptin, $P < 0.06$; interaction, not signficant). Leptin caused a significant reduction in body fat content of AL-fed and 100%-fed control but had no effect in 75%-fed or 125%-fed animals (Fig. 7A). The weights of inguinal, retroperitoneal, and perirenal fat pads were larger in CD than control rats fed the same amount of food, whereas mesenteric and epididymal fat pads were not different (Table 1). The effect of leptin on fat pad size was significant only for inguinal and perirenal fat in 100%-fed CD rats and for inguinal fat in AL-fed controls. Measurement of fat cell size and number in retroperitoneal and epididymal fat confirmed our previous observations (27) that all of the changes in the size of the fat pads were associated with changes in fat cell size rather than any significant change in fat cell number (data not shown). CD rats had

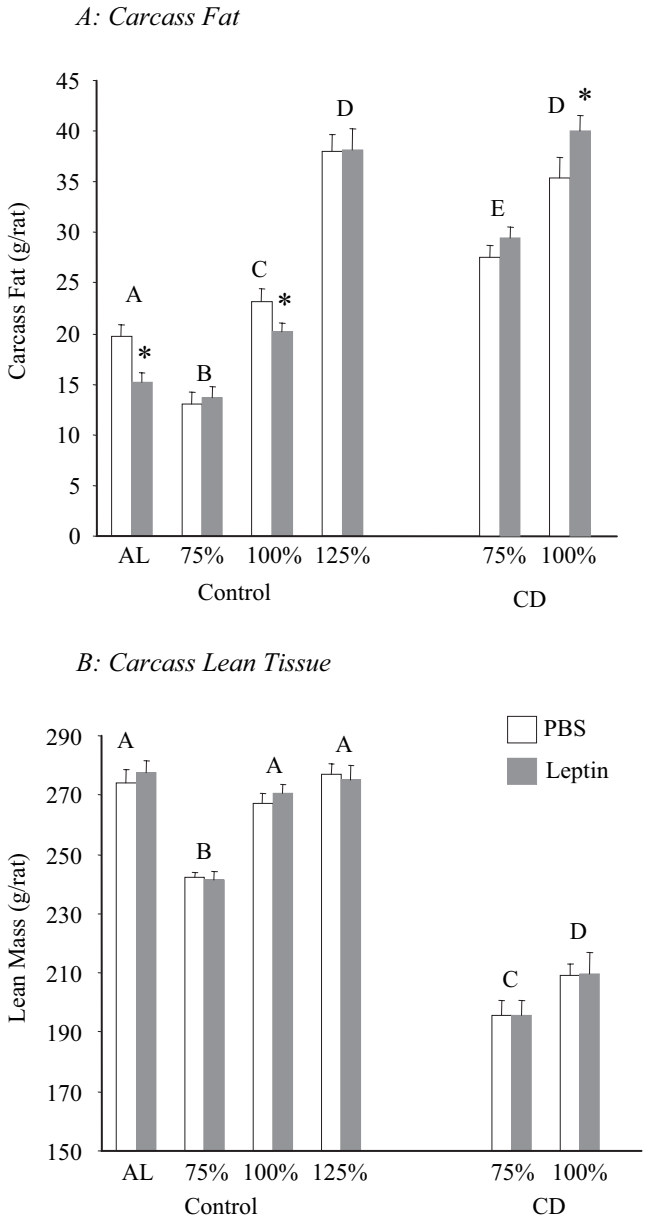

FIG. 7. Carcass fat (A) and lean tissue (protein plus water) (B) measured at the end of the 13-d infusion of leptin or PBS. Data are means + SEM for groups of eight to 11 rats. *Superscript letters* indicate significant ($P < 0.05$) differences between treatment groups, and *asterisks* indicate a significant effect of leptin within a treatment group.

significantly less lean tissue (water plus protein) than intact control rats, and 75%-fed CD rats had significantly less lean tissue than 100%-fed CD rats (Fig. 7B). Lean tissue was significantly reduced in 75%-fed controls compared with other control groups. There was no effect of leptin on lean tissue mass in any treatment group (Fig. 7B). Testes weight was significantly lower in CD than control rats (controls = 3.67 ± 0.03 g; CD = 3.26 ± 0.07 g), but there was no effect of feeding level or of leptin on tissue weight for either CD or control rats.

The weight of IBAT was not different between AL-fed control, 125%-fed control, 75%-fed CD, and 100%-fed CD rats but was reduced in size in 75%-fed and 100%-fed controls

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TABLE 1. Carcass and fat depot weights in control and CD rats

	AL-control		75%-Fed control		100%-Fed control		125%-Fed control		75%-Fed CD		100%-Fed CD	
	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin
Carcass weight (g)	307 ± 3^{a}	306 ± 6^{a}	267 ± 2^{b}	265 ± 2^{b}	301 ± 2^{a}	300 ± 3^{a}	326 ± 3^{c}	325 ± 5^{c}	238 ± 6^{d}	237 ± 8^{d}	250 ± 5^{b}	247 ± 15^{b}
Fat pad weight (mg)												
Inguinal	$2769 \pm 99^{a,c}$	$2408 \pm 136^{a,b,g}$	1990 ± 113^{b}	2118 ± 91^{b}	2949 ± 152^{c}	2928 ± 54^{c}	5147 ± 431^{d}	$4848 \pm 165^{d,e}$	3502 ± 95^{f}	3639 ± 172^{f}	4351 ± 169^{e}	$4770 \pm 350^{d,e,g}$
Epididymal	2412 ± 119	2100 ± 98	1778 ± 61	1893 ± 80	2503 ± 116	2376 ± 103	3020 ± 114	2913 ± 119	1980 ± 64	2019 ± 87	2378 ± 205	2742 ± 123
Retroperitoneal	$977 \pm 75^{a,b,c}$	$800 \pm 96^{a,b}$	646 ± 61^{a}	$699 \pm 85^{a,b}$	1283 ± 106^{c}	$1052 \pm 82^{b,c}$	2117 ± 135^{d}	2037 ± 258^{d}	1233 ± 98^{c}	1297 ± 69^{c}	1611 ± 122^{e}	$1638 \pm 81^{c,d,e}$
Perirenal	$523 \pm 86^{a,b}$	$414 \pm 63^{a,b}$	339 ± 56^{a}	342 ± 34^{a}	$693 \pm 81^{b,c,d}$	$597 \pm 91^{a,c,d}$	$1128 \pm 145^{e,f}$	$1234 \pm 118^{e,f}$	$913 \pm 121^{d,f}$	$974 \pm 112^{d,f}$	958 ± 213^{f}	$1340 \pm 183^{e,g}$
Mesenteric	$2838 \pm 98^{a,c}$	2430 ± 164^{a}	$2179 \pm 229^{a,d}$	$2241 \pm 200^{a,d}$	$3174 \pm 118^{a,d}$	$3040 \pm 224^{a,d}$	4671 ± 166^{b}	4423 ± 240^{b}	$2832 \pm 141^{a,c}$	$2960 \pm 101^{a,c}$	$3971 \pm 343^{b,d}$	$3871 \pm 170^{b,c,d}$
IBAT	431 ± 44^{a}	$353 \pm 27^{a,b}$	233 ± 21^{b}	277 ± 28^{c}	$307 \pm 26^{b,c}$	$298 \pm 34^{b,c}$	$375 \pm 32^{a,b}$	429 ± 18^{a}	$366 \pm 49^{a,b}$	422 ± 32^{a}	$354 \pm 33^{a,b}$	429 ± 58^{a}

Data are means ± SEM for groups of eight to 11 rats. The left inguinal, epididymal, and retroperitoneal pads and all of the perirenal and mesenteric fat was dissected and weighed.

$^{a-f}$ Significant differences between groups. Values that do not share the *same letter* are significantly different at $P < 0.05$.

^{g} Significant ($P < 0.05$) effect of leptin within a treatment group.

(Table 1). Leptin had no effect on the weight of IBAT in any group of rats. Liver weight was significantly greater in CD than control rats, and this was associated with an increase in liver lipid content and in liver glycogen (Table 2). Livers from 100%-fed CD rats were heavier than those from 75%-fed CD rats. Livers from PBS-infused 100%-fed CD rats contained large amounts of glycogen and lipid, but this was reduced to the levels found in 75%-fed CD rats in leptin-infused 100%-fed CD rats. In control rats, liver weight was similar for AL-fed and 100%-fed rats, reduced in 75%-fed rats, and increased in 125%-fed rats. There were no differences in liver lipid based on feeding level, but leptin caused a significant reduction in lipid in 125%-fed controls. Liver glycogen was not different between feeding groups, but leptin increased glycogen in AL-fed controls and decreased glycogen in 125%-fed controls.

The weights of adrenal glands from CD and control rats were the same, and there were no differences in adrenal norepinephrine content, but adrenal epinephrine was lower in CD than control rats (Table 3). There was no effect of feeding level on any of these measures in CD rats, but the adrenals from 75%-fed controls were smaller than those of 125%-fed controls. Serum leptin, measured on d 3 of infusion (Fig. 8A), was increased in both 75%-fed and 100%-fed CD rats compared with their appropriate controls. In control rats, leptin was increased in 125%-fed rats and reduced in 75%-fed rats compared with AL-fed or 100%-fed controls. Leptin infusion caused small increases in circulating concentrations of leptin that reached significance only in the 75%-fed controls and 100%-fed CD rats. Adiponectin, measured at the end of the study, also was higher in CD than control rats and was significantly increased by leptin in the 75%-fed CD rats (Fig. 8B). In control rats, adiponectin was higher in 75%-fed and 125%-fed rats than in AL- or 100%-fed animals, but there was no effect of leptin on adiponectin in any of these groups. Serum testosterone was extremely low in CD rats (Table 4), whereas TSH, corticosterone, and insulin all were in the same range as found in controls. Corticosterone was elevated in both control and CD 75%-fed rats but was significantly lower in 100%-fed CD rats than in any control group. Insulin was lower in both control and CD 75%-fed rats than in any other group. There was no effect of treatment or feeding level on serum glucose concentrations, but CD rats had significantly higher serum triglyceride and glycerol concentrations than control rats (Table 4). Free fatty acids, glycerol, and triglycerides all were higher in 100%-fed than in 75%-fed CD rats. In control rats, triglycerides were higher in 125%-fed rats than in any other group, whereas free fatty acids were significantly lower in 75%-fed rats compared with AL-fed and 125%-fed rats. Glycerol was not influenced by feeding level.

Discussion

The primary objective of this study was to determine whether peripherally infused physiological doses of leptin reduced body fat in rats lacking neural output from the hypothalamus due to the severing of projections between the forebrain and the caudal brainstem, but the control groups allowed us also to determine whether leptin was effective when food intake was controlled and when food intake was fixed above or below voluntary intake. The results show that low-dose peripheral infusions of leptin reduce body fat content of intact control rats even when food intake is fixed but that this response is entirely dependent upon the energy balance status of the rats because neither 75%-fed nor 125%-fed control rats showed any response to leptin infusion. Results from CD rats show, surprisingly, that leptin caused an increase in body fat content. This change was apparent only in the 100%-fed CD animals, implying that it also was dependent upon the energy balance status of the animal. The dependence of leptin responsiveness on energy balance status is not unique to this study because others have shown

TABLE 2. Liver weight and glycogen and lipid content

	AL-control		75%-Fed control		100%-Fed control		125%-Fed control		75%-Fed CD		100%-Fed CD	
	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin	PBS	Leptin
Liver weight (g)	12.6 ± 0.4^{a}	12.7 ± 0.4^{a}	10.3 ± 0.2^{b}	10.3 ± 0.2^{b}	$11.8 \pm 0.2^{a,d}$	11.7 ± 0.3^{d}	13.8 ± 0.2^{c}	13.6 ± 0.4^{c}	10.0 ± 0.4^{b}	9.8 ± 0.3^{b}	11.3 ± 0.2^{d}	$10.9 \pm 0.5^{b,d}$
Glycogen (μg glucose/liver)	$398 \pm 66^{a,c,d}$	$768 \pm 118^{b,e}$	$365 \pm 48^{c,d}$	$372 \pm 60^{c,d}$	$346 \pm 54^{a,c}$	$474 \pm 66^{a,c}$	$643 \pm 81^{b,c}$	$472 \pm 70^{a,c,e}$	206 ± 32^{d}	$310 \pm 33^{c,d}$	725 ± 123^{b}	$264 \pm 42^{d,e}$
Lipid (g/liver)	$0.61 \pm 0.03^{a,b}$	$0.56 \pm 0.04^{a,b}$	0.51 ± 0.02^{a}	0.52 ± 0.02^{a}	$0.57 \pm 0.05^{a,b}$	$0.57 \pm 0.03^{a,b}$	0.69 ± 0.03^{b}	$0.59 \pm 0.02^{a,b,e}$	$0.55 \pm 0.04^{a,b}$	$0.60 \pm 0.03^{a,b}$	0.86 ± 0.16^{c}	$0.64 \pm 0.04^{a,b,e}$

Data are means ± SEM for groups of eight to 11 rats.

$^{a-d}$ Significant differences between groups; groups that do not share *common letters* are significantly different at $P < 0.05$.

^{e} Significant ($P < 0.05$) effect of leptin within a treatment group.

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TABLE 3. Adrenal weight and norepinephrine and epinephrine content

	AL-control	75%-Fed control	100%-Fed control	125%-Fed control	75%-Fed CD	100%-Fed CD
Adrenal weight (mg)	$60 \pm 2^{a,b}$	55 ± 1^{a}	$59 \pm 2^{a,b}$	60 ± 2^{b}	$57 \pm 2^{a,b}$	$56 \pm 2^{a,b}$
Norepinephrine (μg/tissue)	11.7 ± 0.8	11.7 ± 0.7	11.8 ± 0.7	11.2 ± 0.8	10.9 ± 0.7	11.9 ± 0.9
Epinephrine (μg/tissue)	$36.4 \pm 1.5^{a,b}$	38.8 ± 0.9^{a}	38.4 ± 1.2^{a}	37.9 ± 1.2^{a}	33.7 ± 1.8^{b}	33.4 ± 1.6^{b}

Data are means ± SEM for groups of 17–21 rats.
[a] and [b] Values for weight or epinephrine that have *different letters* are significantly different ($P < 0.05$). There was no effect of leptin on any of the measures made for any of the treatment groups.

that food deprivation inhibits leptin transport across the blood-brain barrier in mice (35), that leptin increases hypothalamic expression of neuropeptide Y in rats that have been fasted (36), and that leptin inhibits hyperphagia in rats that are refed after a period of food restriction but does not prevent weight regain and does not inhibit hypothalamic neuropeptide Y mRNA expression in the refed rats (37). The failure of leptin to influence body composition of overfed rats also was not surprising when it is well established that diet-induced obesity induces leptin resistance (38). The leptin response in CD rats could not be attributed to nonspecific outcomes of the brain transection because we have previously shown a normal metabolic response to fasting (27), cold exposure (21), and fourth ventricle stimulation of melanocortin receptors (28).

Previously (27), we reported that CD rats have a lower energy expenditure than intact control rats and are obese after 2 wk of being fed the voluntary intake of control rats. In the present study, we attempted to correct for the difference in energy expenditure by including CD rats that were fed 75% of the voluntary intake of controls. This treatment, however, did not prevent obesity; rather, it caused an even greater inhibition of weight gain and an additional decline in energy expenditure of CD rats. Thus, the increased adiposity of CD rats must result from abnormal regulation of nutrient utilization and metabolism rather than simple positive energy balance. This study confirmed our previous observation that CD rats had very low levels of testosterone, which may be responsible for their abnormal body composition and low metabolic rate. These observations suggest a critical role for the forebrain in regulating reproductive hormones in male rats and are consistent with a previous report that midbrain lesions that sever caudal brainstem-forebrain afferents/efferents compromise testes function (39). Because the rats in this study stopped gaining weight after the first surgery, when only one side of the brain was sectioned, it is possible that even unilateral forebrain disconnection impairs anabolic steroid production. Additional studies are required to determine whether replacement of testosterone would normalize the body composition of CD rats. The low testosterone could have contributed to the high circulating concentrations of leptin in the CD rats, either by increasing body fat mass (40) or by a mechanism that is independent of a change in body fat mass (41), but it does not account for the unexpected increase in body fat of leptin-treated 100%-fed CD rats because testosterone also was low in the PBS-infused CD rats.

Although decerebration resulted in a significant depletion of gonadal steroids, glucocorticoid and adrenal catecholamine synthesis appeared to be within the normal range in CD rats. In addition, because both 75%-fed CD and 75%-fed control rats had elevated levels of corticosterone and because circulating concentrations of TSH were normal, at least some aspects of pituitary function appeared to be intact in the CD rats, although the low testosterone suggests a problem with LH synthesis or with secretion of pituitary gonadotrophs. In a previous study (27), we found that serum T_3 levels were normal in CD rats, and the measurements made here show normal concentrations of TSH, indicating that the low energy expenditure of these rats was not due to impaired thyroid function. In this study, we measured adrenal catecholamine content because Wiater and Ritter (42) reported that adrenal denervation attenuated loss of body fat in rats receiving central infusions of leptin. We did not find any significant change in adrenal weight or norepinephrine content, although there was a significant reduction in adrenal epinephrine content. The procedure of adrenal denervation is likely to have caused a larger change in adrenal catecholamine content than we observed in this study, but the decreased epinephrine content could potentially have contributed to the reduced lean body mass of CD rats because epinephrine inhibits protein degradation in skeletal muscle (43). Others have reported a substantial reduction in adrenal epinephrine content in fasted rats subjected to insulin-induced hypoglycemia (44). In this study, the CD rats were not fasted and had serum insulin and glucose concentrations within the normal range. It is possible, however, that disruption of the neural control of adrenal medullary cells responsible for epinephrine secretion (44) together with an abnormal state of nutrient utilization resulted in a condition that was interpreted at some level as glucoprivation.

The unexpected increase in body fat content of leptin-infused 100%-fed CD rats was associated with a significant decrease in energy expenditure during the light period. We did not measure physical activity in this study, and casual observation suggests that CD rats have low levels of spontaneous activity (45). It should be noted, however, that CD rats are capable of high levels of locomotor activity when they are housed in a cold environment (46). In addition, it is unlikely that leptin would have inhibited spontaneous activity when it has been shown that leptin replacement in leptin-deplete *ob/ob* mice increases activity (3). Therefore, the decrease in energy expenditure was most likely due to a reduction in nonshivering thermogenesis, possibly due to decreased activation of uncoupling proteins in brown adipose tissue. This decrease in thermogenesis is the opposite to the normal effect of leptin on energy expenditure (6, 47), sympathetic outflow to brown adipose tissue, and UCP1 expression in brown adipose tissue of intact rats (47, 48) but is consistent with a previous observation that peripheral administration of lipopolysaccharide decreases IBAT temperature in CD rats but increases IBAT temperature in intact rats (49). These two sets of observations suggest that acti-

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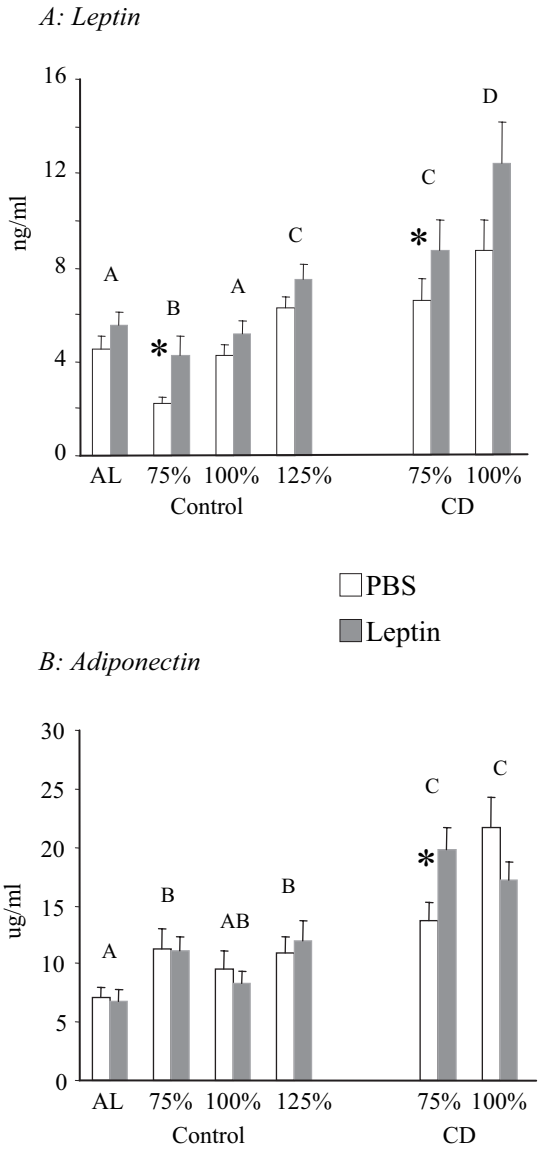

FIG. 8. Serum leptin (A) and adiponectin (B) measured at the end of the 13-d infusion of PBS or leptin. Values are means + SEM for groups of eight to 11 rats. *Letter superscripts* indicate significant ($P < 0.05$) differences between treatment groups, and *asterisks* indicate a significant effect of leptin within a treatment group.

vation of leptin receptors in the caudal brainstem, sympathetic chain, or peripheral tissue inhibits thermogenesis but that this activity is opposed by projections from the forebrain. Severing these projections unveils the inhibitory action of leptin in the hindbrain, reduces energy expenditure, and leads to an increase in body fat in rats that have a fixed energy intake. Energy expenditure of 75%-fed CD rats was already lower than that of the 100%-fed CD rats and was not reduced further by leptin. Serum leptin levels were significantly higher in 100%-fed than 75%-fed CD rats, and it is possible that the inhibition of energy expenditure was produced only by very high leptin concentrations. Alternatively, the 75%-CD rats may have already activated the mechanisms that leptin used to inhibit energy expenditure in 100%-fed CD rats, so that these pathways were not further influenced by leptin.

In contrast to the 100%-fed CD rats, leptin reduced body fat content of both AL-fed and 100%-fed control rats. These data show conclusively that leptin can change body fat in the absence of a change in food intake because intake of the 100%-fed animals was fixed, consistent with previous reports that inhibition of food intake does not account for all of the change in body composition of leptin-treated animals (8, 50). Because the change in body fat was smaller in the 100%-fed than AL-fed rats (13 *vs.* 21%), it is possible that part of the loss in body fat of the AL-fed rats was due to a decrease in food intake that was not detected with 24-h measures. If these rats were in a state of negative energy balance, then one would expect to also find a change in energy expenditure. We did not find any effect of leptin on energy expenditure of the intact rats, which implies that either leptin increased expenditure by an amount that was too subtle to be detected by the 26-h measures of energy expenditure or the loss of fat was due to a change in nutrient partitioning rather than negative energy balance. Others have reported that leptin increases energy expenditure in rats (6, 47), but either leptin was injected into the cerebral ventricles (6) or a high (1 mg/d) dose was infused peripherally (47), which would have resulted in higher concentrations of leptin reaching central leptin receptors than would be expected with the low-dose peripheral infusions used here. In contrast to the 100%-fed rats, leptin had no effect on the body composition of 125%-fed or 75%-fed control rats. It is possible that the already low energy expenditure of the 75%-fed rats prevented any further decrease in thermogenesis or that the inadequate nutrient intake of these animals prevented the change in nutrient partitioning that results in a depletion of body fat. The 125%-fed rats had twice the amount of body fat as the AL-fed rats, and it is possible that this increase in adiposity produced leptin resistance (51). It is important to note that the 125%-fed controls had the same amount of body fat as the 100%-fed CD rats; therefore, the mechanisms that facilitated fat accretion in the CD rats were not available in the 125%-fed controls, presumably due to intact neural communication between the forebrain and caudal brainstem.

Others have reported that leptin reduces body fat by promoting fatty acid oxidation in adipose, muscle, and liver tissue (18, 52–54). If leptin had stimulated fatty acid oxidation, then RER would have decreased toward 0.8, but RER remained at approximately 1.0 in both AL-fed and 100%-fed controls, implying a dependence on carbohydrate oxidation. In CD rats, RER moved from about 0.85 just before a meal to approximately 1.15 after a meal, which would represent a transition from oxidizing fatty acids before the meal to storing energy after the meal. Similar swings in RER were present in 75%-fed control rats, suggesting that 100%-fed CD rats are using ingested nutrients in the normal way. The livers of the CD rats contained more lipid than those of controls, and liver glycogen was very high in the 100%-fed PBS-infused CD rats but very low in the 75%-fed CD rats. It is possible that there is some limitation on their ability to mobilize lipid so that when liver glycogen stores are depleted, the rats start to catabolize protein at a faster rate than would be expected based on their body fat content. This

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TABLE 4. Serum hormones and metabolites

	AL-control	75%-Fed control	100%-Fed control	125%-Fed control	75%-Fed CD	100%-Fed CD
Testosterone (ng/ml)	3.3 ± 0.5^{a}	2.6 ± 0.4^{a}	3.2 ± 0.5^{a}	3.5 ± 0.6^{a}	0.9 ± 0.3^{b}	0.8 ± 0.2^{b}
TSH (μIU/ml)	0.28 ± 0.03	0.32 ± 0.04	0.34 ± 0.04	0.27 ± 0.03	0.29 ± 0.03	0.23 ± 0.03
Corticosterone (ng/ml)	62 ± 12^{a}	215 ± 36^{b}	83 ± 17^{a}	73 ± 18^{a}	140 ± 31^{b}	41 ± 7^{c}
Insulin (ng/ml)	3.5 ± 0.5^{a}	1.8 ± 0.2^{b}	2.1 ± 0.2^{b}	$2.3 \pm 0.2^{a,b}$	1.8 ± 0.2^{b}	$2.5 \pm 0.3^{a,b}$
Glucose (mg/dl)	92 ± 2	94 ± 1	95 ± 3	93 ± 2	93 ± 1	95 ± 2
FFA (nmol/liter)	0.72 ± 0.04^{a}	0.56 ± 0.03^{b}	$0.63 \pm 0.02^{a,b}$	$0.72 \pm 0.04^{a,c}$	$0.66 \pm 0.04^{a,b}$	0.81 ± 0.07^{c}
Triglycerides (mg/dl)	126 ± 8^{a}	122 ± 10^{a}	126 ± 10^{a}	$158 \pm 8^{a,b}$	190 ± 20^{b}	268 ± 38^{c}
Glycerol (ng/ml)	0.28 ± 0.02^{a}	0.27 ± 0.02^{a}	0.27 ± 0.01^{a}	0.32 ± 0.02^{a}	0.44 ± 0.05^{b}	0.61 ± 0.08^{c}

Data are means ± SEM for groups of 17–21 rats.

$^{a-c}$ Values for a particular factor that have *different letters* are significantly different ($P < 0.05$). There was no effect of leptin on any of the serum measures made.

would explain the RER of approximately 0.8 in the postabsorptive state and why the 75%-fed CD rats were much fatter than the 75%-fed control animals. Serum triglycerides, free fatty acids, and glycerol were all elevated in CD rats compared with controls, which would be consistent with increased hepatic lipogenesis and increased turnover of lipids in adipocytes, but additional measurements are needed to determine whether CD rats have a limited ability to use stored lipid for energy and consequently catabolize carbohydrate stores and body protein. The measures of hormone status indicate normal levels of insulin and glucose in CD rats, suggesting that changes in metabolism of CD rats are not due to the development of insulin resistance. In addition, serum adiponectin concentrations were higher in CD than control rats. These results confirm our previous observations (27) but are surprising in that others have reported that adiponectin concentrations are inhibited in conditions of obesity (55). Adiponectin is an adipokine that inhibits hepatic gluconeogenesis, promotes fatty acid oxidation in muscle (56), and is thought to contribute to maintenance of insulin sensitivity in these tissues (57). Because this adipokine acts directly on peripheral tissues, it should remain active in CD rats, and the elevated levels may contribute to their apparently normal insulin responsiveness in the face of obesity.

In summary, the results of this experiment demonstrate that loss of efferent and afferent pathways between the forebrain and hindbrain causes rats to become obese due to changes in energy partitioning rather than a simple decrease in energy expenditure. The reduced lean body mass in these rats may be due to a marked drop in serum testosterone concentrations. The obesity of these rats is associated with elevated levels of leptin and adiponectin but normal blood glucose and insulin concentrations. Peripheral infusions of physiological doses of leptin caused a significant increase in body fat content in these rats that was associated with a decrease in energy expenditure during the light phase of the day. These results imply that neural connections between the forebrain and brain stem normally maintain inhibitory control of leptin-responsive sites in the brainstem, sympathetic chain, or peripheral tissue to combat an inhibition of thermogenesis. Once these neural projections are severed, the inhibitory action of leptin is facilitated and leads to an increase in adiposity. By contrast to the CD rats, peripheral infusions of leptin decreased body fat content of intact control rats that were eating AL or that were tube fed the equivalent of an AL intake. This loss of fat was not associated with a measurable change in either food intake or energy expenditure. The energy status of the animals was a critical determinant of leptin responsiveness because there was no effect of leptin infusion on body composition of 125%-fed controls or of 75%-fed control or CD rats.

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References

1. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
2. **Kelesidis T, Mantzoros CS** 2006 The emerging role of leptin in humans. Pediatr Endocrinol Rev 3:239–248
3. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540–543
4. **Farooqi S, Rau H, Whitehead J, O'Rahilly S** 1998 ob gene mutations and human obesity. Proc Nutr Soc 57:471–475
5. **Harris RB, Zhou J, Redmann Jr SM, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ** 1998 A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. Endocrinology 139:8–19
6. **Wang T, Hartzell DL, Rose BS, Flatt WP, Hulsey MG, Menon NK, Makula RA, Baile CA** 1999 Metabolic responses to intracerebroventricular leptin and restricted feeding. Physiol Behav 65:839–848
7. **Hwa JJ, Fawzi AB, Graziano MP, Ghibaudi L, Williams P, Van Heek M, Davis H, Rudinski M, Sybertz E, Strader CD** 1997 Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. Am J Physiol 272:R1204–R1209
8. **Bowen H, Mitchell TD, Harris RB** 2003 Method of leptin dosing, strain, and group housing influence leptin sensitivity in high-fat-fed weanling mice. Am J Physiol Regul Integr Comp Physiol 284:R87–R100
9. **Penn DM, Jordan LC, Kelso EW, Davenport JE, Harris RB** 2006 The effects of central or peripheral leptin administration on norepinephrine turnover in defined fat depots. Am J Physiol Regul Integr Comp Physiol 291:R1613–R1621
10. **Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM** 2001 Selective deletion of leptin receptor in neurons leads to obesity. J Clin Invest 108:1113–1121
11. **Shek EW, Scarpace PJ** 2000 Resistance to the anorexic and thermogenic effects of centrally administrated leptin in obese aged rats. Regul Pept 92:65–71
12. **Commins SP, Watson PM, Frampton IC, Gettys TW** 2001 Leptin selectively reduces white adipose tissue in mice via a UCP1-dependent mechanism in brown adipose tissue. Am J Physiol Endocrinol Metab 280:E372–E377

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13. **Bagnasco M, Dube MG, Kalra PS, Kalra SP** 2002 Evidence for the existence of distinct central appetite, energy expenditure, and ghrelin stimulation pathways as revealed by hypothalamic site-specific leptin gene therapy. Endocrinology 143:4409–4421
14. **Bamshad M, Song CK, Bartness TJ** 1999 CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am J Physiol 276:R1569–R1578
15. **Tartaglia LA** 1997 The leptin receptor. J Biol Chem 272:6093–6096
16. **Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J** 1995 Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263–1271
17. **Harris RB, Mitchell TD, Yan X, Simpson JS, Redmann SM, Jr.** 2001 Metabolic responses to leptin in obese db/db mice are strain dependent. Am J Physiol Regul Integr Comp Physiol 281:R115–R132
18. **Huang W, Dedousis N, Bandi A, Lopaschuk GD, O'Doherty RM** 2006 Liver triglyceride secretion and lipid oxidative metabolism are rapidly altered by leptin *in vivo*. Endocrinology 147:1480–1487
19. **Huang W, Dedousis N, Bhatt BA, O'Doherty RM** 2004 Impaired activation of phosphatidylinositol 3-kinase by leptin is a novel mechanism of hepatic leptin resistance in diet-induced obesity. J Biol Chem 279:21695–21700
20. **Grill HJ, Kaplan JM** 2002 The neuroanatomical axis for control of energy balance. Front Neuroendocrinol 23:2–40
21. **Grill HJ** 2006 Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. Obesity 14(Suppl 5):216S–221S
22. **Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG** 2002 Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology 143:239–246
23. **Huo L, Grill HJ, Bjorbaek C** 2006 Divergent regulation of proopiomelanocortin neurons by leptin in the nucleus of the solitary tract and in the arcuate hypothalamic nucleus. Diabetes 55:567–573
24. **Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ** 1998 Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. Am J Physiol 275:R291–R299
25. **Cao WH, Fan W, Morrison SF** 2004 Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience 126:229–240
26. **Ootsuka Y, McAllen RM** 2005 Interactive drives from two brainstem premotor nuclei are essential to support rat tail sympathetic activity. Am J Physiol Regul Integr Comp Physiol 289:R1107–R1115
27. **Harris RB, Kelso EW, Flatt WP, Bartness TJ, Grill HJ** 2006 Energy expenditure and body composition of chronically maintained decerebrate rats in the fed and fasted condition. Endocrinology 147:1365–1376
28. **Williams DL, Bowers RR, Bartness TJ, Kaplan JM, Grill HJ** 2003 Brainstem melanocortin 3/4 receptor stimulation increases uncoupling protein gene expression in brown fat. Endocrinology 144:4692–4697
29. **Harris RB, Martin RJ** 1989 Changes in lipogenesis and lipolysis associated with recovery from reversible obesity in mature female rats. Proc Soc Exptl Biol Med 191:82–89
30. **Marques BG, Hausman DB, Martin RJ** 1998 Association of fat cell size and paracrine growth factors in development of hyperplastic obesity. Am J Physiol 275:R1898–R1908
31. **Harris RB** 1994 Factors influencing energy intake of rats fed either a high-fat or a fat mimetic diet. Intl J Obes Relat Metab Dis 18:632–640
32. **Harris RB** 1991 Growth measurements in Sprague-Dawley rats fed diets of very low fat concentration. J Nutr 121:1075–1080
33. **McClave SA, Lowen CC, Kleber MJ, McConnell JW, Jung LY, Goldsmith LJ** 2003 Clinical use of the respiratory quotient obtained from indirect calorimetry. J Parenter Enteral Nutr 27:21–26
34. **Leveille GA, O'Hea EK** 1967 Influence of periodicity of eating on energy metabolism in the rat. J Nutr 93:541–545
35. **Kastin AJ, Akerstrom V** 2000 Fasting, but not adrenalectomy, reduces transport of leptin into the brain. Peptides 21:679–682
36. **Bertile F, Oudart H, Maho YL, Raclot T** 2003 Recombinant leptin in the hypothalamic response to late fasting. Biochem Biophys Res Commun 310:949–955
37. **Velkoska E, Morris MJ, Burns P, Weisinger RS** 2003 Leptin reduces food intake but does not alter weight regain following food deprivation in the rat. Int J Obes Relat Metab Disord 27:48–54
38. **Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, Cowley MA** 2007 Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab 5:181–194
39. **Ali MA** 1993 Effects of mesencephalic lesion on testes, seminal vesicles and adrenal glands of albino rats. Indian J Physiol Pharmacol 37:97–103
40. **Baltaci AK, Mogulkoc R, Ozturk A** 2006 Testosterone and zinc supplementation in castrated rats: effects on plasma leptin levels and relation with LH, FSH and testosterone. Life Sci 78:746–752
41. **Page S, Herbst K, Amory J, Coviello A, Anawalt B, Matsumoto A, Bremner W** 2005 Testosterone administration suppresses adiponectin levels in men. J Androl 26:85–92
42. **Wiater M, Ritter S** 2005 Sympathoadrenal and sympathetic contributions to central leptin-induced fat loss. Proc North American Society for the Study of Obesity Annual Meeting, Vancouver, British Columbia, Canada, 2005 (Abstract 9-HT)
43. **Tischler ME** 1981 Hormonal regulation of protein degradation in skeletal and cardiac muscle. Life Sci 28:2569–2576
44. **Vollmer RR, Baruchin A, Kolibal-Pegher SS, Corey SP, Stricker EM, Kaplan BB** 1992 Selective activation of norepinephrine- and epinephrine-secreting chromaffin cells in rat adrenal medulla. Am J Physiol 263:R716–R721
45. **Grill HJ, Norgren R** 1978 Neurological tests and behavioral deficits in chronic thalamic and chronic decerebrate rats. Brain Res 143:299–312
46. **Grill H, Nautiyal K, Kaplan J** 2005 Thermoregulatory responses to cold in chronic decerebrate rats. Society for Neuroscience Abstract Viewer Itinerary Planner:Program number 183.23
47. **Scarpace PJ, Matheny M, Pollock BH, Tumer N** 1997 Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 273:E226–E230
48. **Scarpace PJ, Matheny M** 1998 Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. Am J Physiol 275:E259–E264
49. **Williams D, Sadacca L, Carmody J, Kaplan J, Grill H** 2004 Dissociation of ingestive and thermal responses to lipopolysaccharide in chronic decerebrate rats. Appetite 42:410
50. **Levin N, Nelson C, Gurney A, Vandlen R, de Sauvage F** 1996 Decreased food intake does not completely account for adiposity reduction after ob protein infusion. Proc Natl Acad Sci USA 93:1726–1730
51. **Enriori PJ, Evans AE, Sinnayah P, Cowley MA** 2006 Leptin resistance and obesity. Obesity 14(Suppl 5):254S–258S
52. **Wang MY, Lee Y, Unger RH** 1999 Novel form of lipolysis induced by leptin. J Biol Chem 274:17541–17544
53. **Muoio DM, Dohn GL, Fiedorek Jr FT, Tapscott EB, Coleman RA** 1997 Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 48:1360–1363
54. **Minokoshi Y, Kahn BB** 2003 Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem Soc Trans 31:196–201
55. **Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C, Mantzoros CS** 2003 Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab 88:4823–4831
56. **Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T** 2002 Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295
57. **Yamauchi T, Hara K, Kubota N, Terauchi Y, Tobe K, Froguel P, Nagai R, Kadowaki T** 2003 Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. Curr Drug Targets Immune Endocr Metabol Disord 3:243–254

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