

Role of Selective Leptin Resistance in Diet-Induced Obesity Hypertension

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Leptin is an adipocyte-derived hormone that plays a key role in the regulation of body weight through its actions on appetite and metabolism. Leptin also increases sympathetic nerve activity (SNA) and blood pressure. We tested the hypothesis that diet-induced obesity is associated with resistance to the metabolic actions of leptin but preservation of its renal SNA and arterial pressure effects, leading to hypertension. Mice were fed a high-fat diet for 10 weeks to induce moderate obesity. The decrease in food intake and body weight induced by intraperitoneal or intracerebroventricular leptin was significantly attenuated in the obese mice. Regional SNA responses to leptin were differentially altered in diet-induced obese mice. Renal SNA response to leptin was preserved, whereas lumbar and brown adipose tissue SNA responses were attenuated in obese mice. Radiotelemetric arterial pressure was ~10 mmHg higher in obese mice. Furthermore, the increase in arterial pressure in response to long-term (12 days) leptin treatment was preserved in obese mice. Thus, mice with diet-induced obesity exhibit circulating hyperleptinemia and resistance to the metabolic actions of leptin. However, there is preservation of the renal sympathetic and arterial pressure responses to leptin, which represent a potential mechanism for the adverse cardiovascular consequences of obesity. *Diabetes* 54: 2012–2018, 2005

Obesity is an important risk factor for cardiovascular morbidity and mortality. The mechanisms linking obesity and cardiovascular disorders remain poorly understood. Several mechanisms have been implicated, including overactivity of the sympathetic nervous system (1–3). Recent evidence indicates that leptin may play a role in the high arterial pressure associated with excess weight gain. Leptin, a 167–amino acid protein secreted by adipocytes (4), relays a satiety signal to the central nervous system where it acts to decrease appetite and increase energy expenditure

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BAT, brown adipose tissue; SNA, sympathetic nerve activity.

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through activation of the sympathetic nerve outflow to thermogenic tissues, such as the brown adipose tissue (BAT) (5). Leptin also causes a significant and dose-dependent sympathetic excitation to nonthermogenic tissues, such as the kidneys and adrenal glands (5,6). Renal sympathetic activation to leptin suggests that this hormone plays a role in cardiovascular regulation. Indeed, chronic infusion and transgenic overexpression of leptin has been shown to increase arterial blood pressure and heart rate (7,8). In contrast, leptin deficiency, as in the *ob/ob* mouse, leads to decreased arterial pressure, despite severe obesity (9).

Plasma leptin levels are significantly elevated in obese individuals compared with lean subjects (10,11). Therefore, it has been suggested that most obese subjects have resistance to the anorectic and weight-reducing effects of leptin. Emerging evidence suggest that leptin resistance associated with obesity may be selective and spare some actions of leptin. For example, in agouti obese mice, there is resistance to the metabolic effects of leptin, but leptin still contributes to the hypertension observed in this model (8,9). Recently, we have demonstrated that agouti obese mice have selective leptin resistance with preserved renal sympathetic activation despite the loss of the anorectic and weight-reducing effects of leptin. The effects of leptin on food intake and body weight were significantly less in agouti obese mice than in lean controls, whereas the increase in renal sympathetic nerve outflow was preserved in agouti obese mice (12,13). However, the relevance of this mouse model to human obesity is unclear, as no form of human obesity caused by overexpression of agouti or agouti-related proteins has been reported.

In the present study, we examined the role of leptin in hypertension associated with an acquired form of obesity, namely diet-induced obesity. Dietary models of obesity are widely accepted models for common human obesity. This is based on the observation that diet-induced obesity in animal models recapitulates the features of obesity in humans, including metabolic and cardiovascular changes (14). We tested the hypothesis that diet-induced obesity is associated with resistance to the metabolic actions of leptin but preservation of the cardiovascular sympathetic actions, leading to hypertension.

RESEARCH DESIGN AND METHODS

Male C57BL/6J mice, at the age of 5–6 weeks, were obtained from The Jackson Laboratories (Bar Harbor, ME). Mice were weighed and randomly assigned to normal diet (Harlan Teklad, Madison, WI) or high-fat diet (D12451; Research Diets, New Brunswick, NJ). Normal diet was composed of protein (18.62%), fat (6.25%), fibers (4.53%), and nitrogen-free extracts (53%). High-fat diet

consisted of a special diet composed of protein (24%), fat (24%), and carbohydrate (41%). Body weight was recorded every week for 10 weeks. Mice were housed in a room with constant temperature (23°C) and a 12-h light/dark cycle (light turned off at 6 P.M.) with free access to food (normal or high-fat diet) and tap water. The University of Iowa Animal Research Committee approved all protocols.

Procedures

Recording of sympathetic nerve activity. Mice were anesthetized by an intraperitoneal injection of ketamine (91 mg/kg) and xylazine (9.1 mg/kg). To measure direct multifiber regional sympathetic nerve activity (SNA), a nerve fascicle to the left kidney, hindlimb, or BAT was carefully isolated. A bipolar platinum-iridium electrode (Cooner Wire) was suspended under the nerve and secured with silicone gel (Kwik-Cast; WPI, Sarasota, FL). The nerve signal was amplified and filtered as previously described (12,13). The integrated voltage after death (background noise) was subtracted from the total integrated voltage to calculate real sympathetic activity for each nerve.

Measurement of arterial pressure. Arterial pressure was recorded in mice using continuous radiotelemetric measurement. Mice were intraperitoneally anesthetized with a ketamine/xylazine cocktail. The left common carotid artery was isolated, and the catheter was inserted and tied securely using the silk. The transmitter was slipped under the skin and down into a dissected free "pocket" along the flank as close to the right hindlimb as possible. The neck incision was closed using silk and further sealed with tissue adhesive. Mice were kept warm on a heating pad and monitored closely until fully recovered from anesthesia.

Intracerebroventricular cannulation. Intracerebroventricular cannulae implantation was performed as previously described (12). Briefly, mice were anesthetized by an intraperitoneal injection of ketamine/xylazine cocktail and placed in a stereotaxic device (Kopf Instruments), and a cannula was implanted into the right lateral brain ventricle. Mice were given 5–7 days to recover from surgery.

Plasma leptin assay. Plasma was obtained by centrifuging (5,000 rpm for 5 min) blood collected from mice. Plasma concentration of murine leptin was measured by radioimmunoassay using a commercially available kit (Linco).

Effects of leptin on food intake and body weight. Body weight and food intake of individually caged lean and obese mice were measured daily for 3 consecutive days before treatment. Mice ($n = 15$ – 16 animals per group) were then assigned to receive intraperitoneal murine leptin (R & D Systems) at 30 or 60 μg or vehicle (0.9% NaCl, 30 μl) twice daily. Body weight and food intake were measured daily for 3 consecutive days during leptin or vehicle treatment. Other groups of mice on normal or high-fat diet were assigned to receive one intracerebroventricular injection of leptin (5 μg) or vehicle (2 μl). Body weight and food intake were measured 24 h after intracerebroventricular leptin or vehicle. We weighed three fat pads (BAT and epididymal and perirenal fat) when each mouse was killed.

Design

Effects of leptin on regional SNA. Anesthetized mice were instrumented for measurement of arterial pressure and heart rate (via a catheter inserted in the carotid artery) and SNA to the kidney, hindlimb, or BAT. A catheter was inserted into the jugular vein for maintenance of anesthesia with intravenous α -chloralose (25 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Body temperature was maintained at 37.5°C with the assistance of a lamp and a heating pad. SNA and hemodynamic parameters were recorded at baseline (10 min) and during 240 min after treatment.

The effect of intravenous leptin and vehicle on renal, BAT, and lumbar SNA was compared between mice fed normal or high-fat diet (with 10–17 animals per group). A higher dose of intravenous leptin was used for lumbar and BAT SNA (60 and 120 μg) compared with renal SNA (30 and 60 μg), because these nerves were found to be less sensitive to leptin. In a subset of mice fed normal ($n = 6$) or high-fat ($n = 6$) diet, renal nerves were cut distally to the site of recording before intravenous administration of leptin (60 μg).

The responses of renal, BAT, and lumbar SNA to intracerebroventricular leptin (5 μg) and vehicle (2 μl) were also compared between mice fed normal or high-fat diet (with 5–16 animals per group). At the end of the study, blood samples were collected from the carotid artery for plasma leptin measurements. Finally, animals were killed with a lethal dose of ketamine/xylazine.

Effects of obesity and leptin on arterial pressure. After 1 week of recovery from surgery to implant the radiotelemetry transmitters, arterial pressure and heart rate were recorded in the conscious unrestrained state for 7 days. Lean and obese mice ($n = 7$ – 8 animals per group) then received 12 days treatment with vehicle or leptin (60 μg i.p., twice daily) with continued arterial pressure and heart rate recordings. Body weight and food intake were measured daily before and after starting the leptin treatment. Because leptin did not significantly decrease food intake in the obese mice, a pair-fed group

of mice was included only for the mice on normal diet. Pair-fed mice ($n = 6$) received a daily amount of food equal to the amount of food consumed by the lean mice treated with leptin. These pair-fed mice also received intraperitoneal vehicle treatment.

Data analysis. Results are expressed as means \pm SE. Because of the animal-to-animal variability in baseline SNA, the data for SNA are expressed as percentage change from baseline. Data were analyzed using one- or two-way ANOVA. When analysis of ANOVA reached significance, the Student-Newman-Keuls (when there is an equal number of animals in each group) or Bonferroni (when the number of animals differs between groups) tests were used to compare the mean values among the different levels of mice diets and treatments. A value of $P < 0.05$ was considered significant.

RESULTS

Obesity was induced in C57BL/6J mice by exposure to a diet in which 45% of the calories were derived from fat ($n = 287$); a parallel group of mice was fed a normal diet with 13% fat ($n = 250$). While body weight was comparable at baseline (17.3 ± 0.2 and 17.4 ± 0.1 g in mice on normal or high-fat diet, respectively), mice on the high-fat diet exhibited a greater weight gain than the controls on normal diet ($P < 0.001$, Fig. 1A). The difference in body weight became statistically significant as early as 1 week after dietary treatment (19.7 ± 0.1 g in mice on normal diet and 20.5 ± 0.1 g in mice on high-fat diet, $P < 0.001$). After 10 weeks, mice on high-fat diet weighed $\sim 10\%$ more than those fed the normal diet. Weight of fat pads was also significantly higher in the mice on high-fat diet (Fig. 1B–D) as was the plasma concentration of leptin (see vehicle-treated groups in Tables 1 and 2).

Systemic effects of leptin in mice on normal or high-fat diet

Food intake and body weight responses to leptin. Intraperitoneal administration of leptin caused a dose-dependent suppression of cumulative food intake ($P < 0.001$) and weight loss ($P < 0.001$) in mice on normal diet (Fig. 2). As expected, mice on high-fat diet were substantially resistant to the anorexic effects of leptin. Leptin produced a significant decrease in food intake ($P = 0.039$) in mice fed high fat, but it was significantly less ($P = 0.006$) than in mice fed normal diet. Furthermore, whereas leptin decreased body weight in mice fed normal diet, there was no significant decrease in body weight in mice fed high-fat diet (Fig. 2A). Leptin treatment was associated with a decrease in BAT weight in mice on normal diet but not in high-fat-fed mice (Table 1). Epididymal fat and perirenal fat depot weights were not significantly affected by leptin in both groups of mice (Table 1).

Regional SNA responses to leptin. At baseline (before intravenous treatment), no difference in basal renal SNA was observed between mice fed normal diet (2.98 ± 0.08 volts $\cdot \text{s}^{-1} \cdot \text{min}^{-1}$) or high-fat diet (2.96 ± 0.08 volts $\cdot \text{s}^{-1} \cdot \text{min}^{-1}$). Baseline lumbar and BAT SNA were also comparable between mice fed normal or high-fat diet (data not shown).

Intravenous administration of similar doses of leptin as above (30 and 60 μg) caused substantial increases in SNA to the kidney in mice fed normal diet ($P < 0.001$) or high-fat diet ($P < 0.001$) (Fig. 3). There was no significant difference in effects of leptin on renal SNA between mice on normal or high-fat diet ($P = 0.683$). Intravenous administration of 60 μg leptin increased renal SNA by 222 ± 29 and $244 \pm 27\%$ in the 4th h in mice on normal or high-fat diet, respectively.

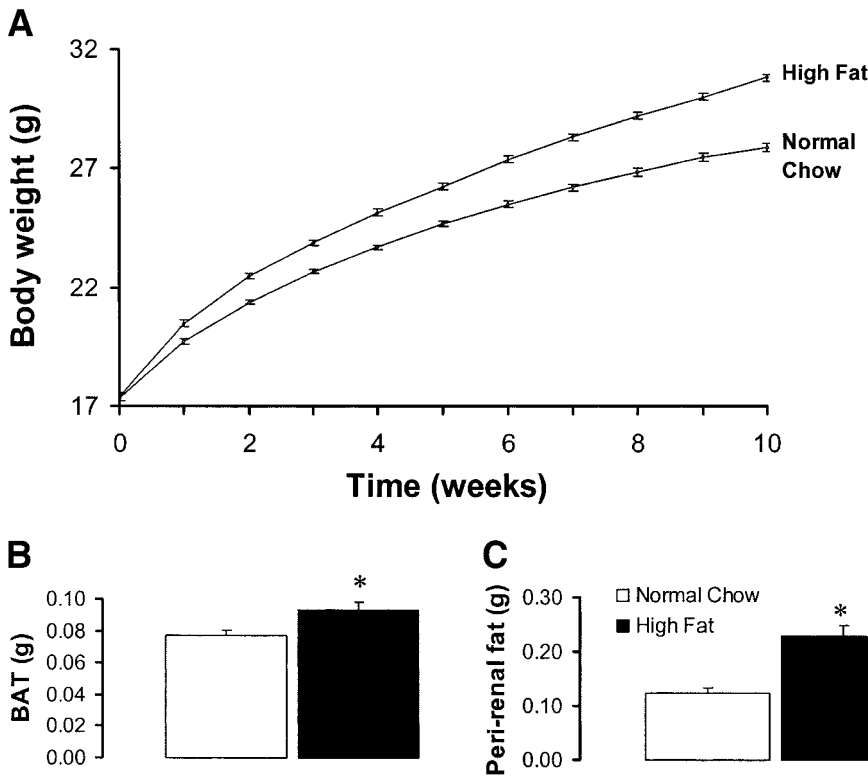


FIG. 1. Obesity development in mice fed high-fat diet. **A**: Weekly body weight of mice fed normal diet ($n = 250$) or high-fat diet ($n = 287$) for 10 weeks. **B–D**: Weight of BAT, peri-renal fat, and epididymal fat in mice on normal or high-fat diet for 10 weeks. Data are means \pm SE. * $P < 0.05$ vs. mice on normal diet.

Transection of the renal nerve distal to the recording site did not affect the renal sympathetic activation to leptin, with an increase in renal SNA of $232 \pm 19\%$ ($n = 6$) and $232 \pm 30\%$ ($n = 6$) in the 4th h of $60 \mu\text{g}$ intravenous leptin in mice on normal or high-fat diet, respectively. In contrast to renal SNA, the increases in SNA to BAT and hindlimb following intravenous leptin were markedly attenuated ($P = 0.003$ and $P < 0.001$, respectively) in mice fed high-fat diet compared with mice on normal diet (Fig. 4). Intravenous administration of leptin increased plasma leptin concentrations identically in mice on both diets (Table 1).

TABLE 1
Plasma leptin and weight of different fat depots in mice on normal or high-fat diet treated with either intravenous vehicle or leptin

Mice diet	Vehicle	Leptin	
		30 μg	60 μg
Normal diet			
Plasma leptin (ng/ml)	5 ± 1	$56 \pm 8^*$	$94 \pm 14^*$
BAT (g)	0.099 ± 0.005	$0.073 \pm 0.003^*$	$0.073 \pm 0.003^*$
Epididymal fat (g)	0.570 ± 0.057	0.479 ± 0.051	0.490 ± 0.058
Perirenal fat (g)	0.175 ± 0.023	0.139 ± 0.016	0.139 ± 0.024
High-fat diet			
Plasma leptin (ng/ml)	$11 \pm 3^\dagger$	64 ± 14	104 ± 18
BAT (g)	0.104 ± 0.011	$0.091 \pm 0.008^\dagger$	0.097 ± 0.013
Epididymal fat (g)	0.816 ± 0.120	$0.836 \pm 0.130^\dagger$	$1.046 \pm 0.141^\dagger$
Perirenal fat (g)	0.231 ± 0.042	$0.239 \pm 0.039^\dagger$	$0.302 \pm 0.043^\dagger$

Data are means \pm SE of 15–16 animals per group. * $P < 0.05$ vs. vehicle, $^\dagger P < 0.05$ vs. mice on normal diet.

Central effects of leptin in mice on normal or high-fat diet

Food intake and body weight responses to intracerebroventricular leptin. In mice on normal diet, intracerebroventricular injection of $5 \mu\text{g}$ leptin caused a significant decrease in food intake ($P < 0.001$, Fig. 5A) and body weight ($P = 0.001$) measured 24 h after the intracerebroventricular injection. However, in high-fat-fed mice, intracerebroventricular leptin did not significantly affect food intake (Fig. 5A, $P = 0.081$) or body weight ($P = 0.594$), compared with the vehicle. In normal diet-fed mice, the decrease in body weight induced by intracerebroventricular leptin was associated with a decrease ($P = 0.009$) in BAT mass measured 24 h after the intracerebroventricular injection (Table 2). Epididymal and perirenal fat did not change significantly in the normal diet-fed mice

TABLE 2
Plasma leptin and weight of different adipose depots in mice on normal or high-fat diet treated with intracerebroventricular vehicle or leptin

Mice diet	Vehicle	Leptin (5 μg)
Normal diet		
Plasma leptin (ng/ml)	3 ± 1	5 ± 1
BAT (g)	0.074 ± 0.002	$0.058 \pm 0.003^*$
Epididymal fat (g)	0.292 ± 0.022	0.231 ± 0.029
Perirenal fat (g)	0.080 ± 0.006	0.068 ± 0.010
High-fat diet		
Plasma leptin (ng/ml)	$13 \pm 3^\dagger$	$19 \pm 3^\dagger$
BAT (g)	$0.083 \pm 0.005^\dagger$	$0.079 \pm 0.004^\dagger$
Epididymal fat (g)	$0.721 \pm 0.113^\dagger$	$0.513 \pm 0.063^\dagger$
Perirenal fat (g)	$0.211 \pm 0.032^\dagger$	$0.196 \pm 0.059^\dagger$

Data are means \pm SE of 15–16 animals per group. * $P < 0.05$ vs. vehicle, $^\dagger P < 0.05$ vs. mice on normal diet.

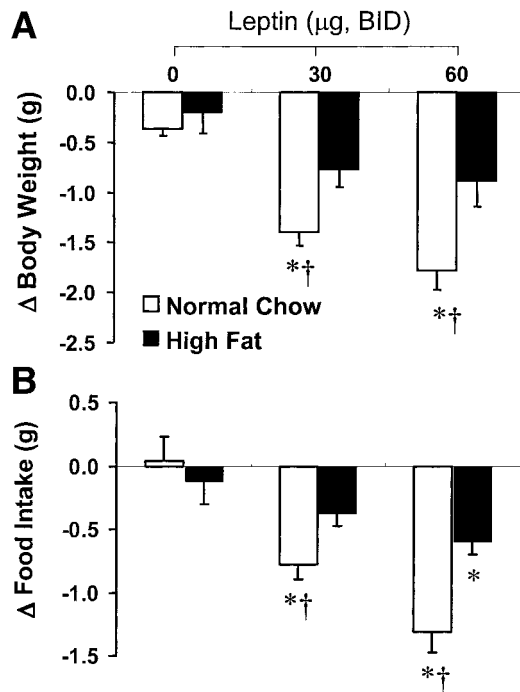


FIG. 2. Change in body weight (A) and food intake (B) after intraperitoneal administration of vehicle or leptin (30 and 60 µg, twice daily for 3 days) in mice on normal or high-fat diets. Data are means \pm SE of 15–16 animals per group. There was a significant difference between mice on normal or high-fat diet in the effects of leptin on body weight ($P < 0.001$) and food intake ($P = 0.006$). * $P < 0.05$ vs. vehicle, † $P < 0.05$ vs. mice on high fat diet.

after intracerebroventricular leptin (Table 2). In the high-fat-fed mice, no fat pad depot was affected by intracerebroventricular leptin compared with the vehicle (Table 2). Plasma levels of leptin (measured 24 h after treatment) were not affected by intracerebroventricular administration of leptin in mice on both diets (data not shown).

Regional SNA responses to intracerebroventricular leptin. The rise in renal SNA induced by intracerebroventricular leptin was of the same magnitude in mice on normal or high-fat diet ($P = 0.917$, Fig. 5B). Indeed, the increase in renal SNA in the 4th h after intracerebroventricular leptin was 144 ± 14 and $145 \pm 15\%$ in mice on normal or high-fat diet, respectively.

In accordance with the response to intravenous treatment, BAT sympathetic activation to intracerebroventricular leptin was significantly attenuated ($P = 0.029$) in mice on high-fat diet compared with normal diet-fed mice (Fig. 5C). Lumbar SNA response to intracerebroventricular leptin was also attenuated ($P = 0.05$) in mice on high-fat diet compared with mice fed normal diet (Fig. 5D). Again, plasma levels of leptin (measured here 4 h after treatment) were not affected by intracerebroventricular administration of leptin in mice on both diets (Table 2).

Blood pressure measurements

Effect of dietary obesity on arterial pressure. We compared arterial pressure between mice on normal or high-fat diet. Using radiotelemetric measurement, mice on high-fat diet showed ~ 10 mmHg higher baseline systolic, diastolic, and mean arterial pressure than the mice on normal diet. Mean arterial pressure was 110 ± 1 mmHg in mice fed high-fat diet vs. 100 ± 1 mmHg in control mice ($P < 0.001$). In contrast to arterial pressure, heart rate was

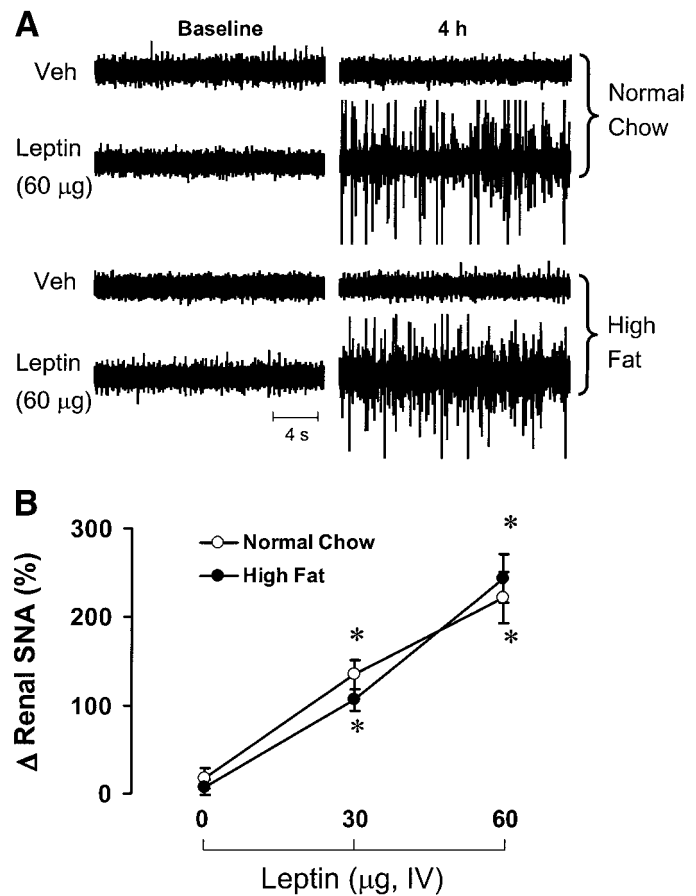


FIG. 3. Effect of bolus intravenous administration of vehicle and leptin on renal SNA in mice on normal or high-fat diet. A: Segments of original records of renal SNA at baseline and 4 h after vehicle or leptin (60 µg). B: Average of the 4th h of renal SNA responses to vehicle and leptin (30 and 60 µg). Data are means \pm SE of 15 animals per group. There was no significant difference between mice on normal or high-fat diet in the effects of leptin on renal sympathetic outflow ($P = 0.683$). * $P < 0.01$ vs. vehicle.

significantly lower in mice on high-fat diet (385 ± 3 bpm) compared with controls (417 ± 3 bpm, $P < 0.01$).

Effect of leptin on arterial pressure in mice on normal or high-fat diet. The question here was whether there is resistance or preservation of the arterial pressure response to leptin in obese mice fed high-fat diet. The preserved renal SNA response to leptin associated with the hyperleptinemia observed in mice on high-fat diet might be expected to increase arterial pressure in these obese mice. In line with our previous reports (12,13), short-term intravenous or intracerebroventricular leptin treatments in anesthetized mice caused no significant changes in arterial pressure or heart rate recorded during the regional SNA experiments (data not shown). To test the ability of long-term leptin treatment to increase arterial pressure in the obese mice despite the resistance to the anorectic and weight-reducing effect of leptin, we compared the action of 12 days intraperitoneal administration of vehicle and leptin (60 µg, twice daily) in mice on normal or high-fat diet. In mice on normal diet, leptin caused a significant ($P < 0.001$) increase in mean arterial pressure as expected (Fig. 6A). Heart rate did not change significantly ($P = 0.331$) in leptin-treated (Δ heart rate at day 12 was $+15 \pm 7$ bpm) and vehicle-treated ($+5 \pm 9$ bpm)

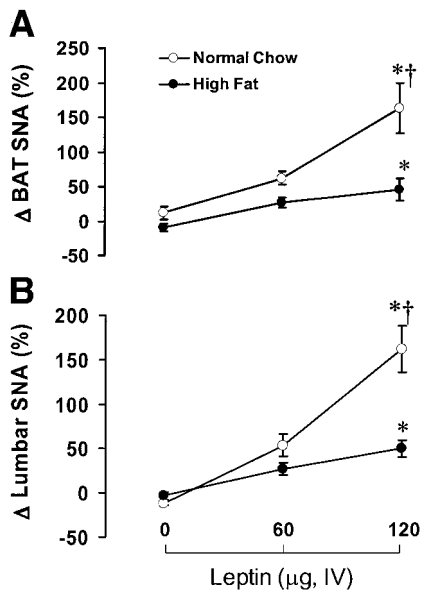


FIG. 4. Effect of bolus intravenous administration of vehicle and leptin (60 and 120 μ g) on sympathetic nerve activity (SNA) to BAT (A) and hindlimb (B) in mice on normal or high-fat diet. Data are means \pm SE of 10–17 animals per group and represent the average of the last hour of SNA responses. There was a significant difference between mice on normal or high-fat diet in the effects of leptin on BAT ($P = 0.003$) and lumbar SNA ($P < 0.001$). * $P < 0.01$ vs. vehicle, † $P < 0.05$ vs. mice on high-fat diet.

groups. Leptin treatment also caused a significant decrease in body weight ($-6.0 \pm 0.9\%$), food intake ($-32.4 \pm 7.5\%$), and fat mass (-3 to -21%). Arterial pressure in the pair-fed lean mice treated with vehicle (Δ mean arterial pressure at day 12 was -3 ± 8 mmHg, $n = 6$) did not change significantly ($P = 0.2$) compared with the nonpair-fed vehicle-treated group (-2 ± 2 mmHg).

Arterial pressure in mice on high-fat diet was also responsive to leptin. Twelve days of leptin caused a significant increase in arterial pressure in the high-fat-fed mice (Fig. 6B). The leptin-induced arterial pressure increase was of the same magnitude in mice fed high-fat diet (~ 10 mmHg) or normal diet (~ 11 mmHg). Leptin caused no significant change in heart rate ($+14 \pm 4$ bpm), body weight ($-2.7 \pm 0.9\%$), and food intake ($-1.1 \pm 9.9\%$) in mice on high-fat diet.

DISCUSSION

We previously reported that agouti yellow obese mice have selective leptin resistance with preservation of the renal sympathetic response to leptin despite resistance to the anorectic and weight-reducing actions of leptin. The present study extends these observations in three ways. First, our data show selective leptin resistance in an acquired model of obesity, namely mice with diet-induced obesity. Second, the study demonstrates that there is preservation of the arterial pressure response to leptin in addition to the renal sympathetic response to leptin in this model of obesity hypertension. This finding enhances the potential pathophysiologic significance of the phenomenon of selective leptin resistance. Third, the study demonstrates that preservation of the sympathetic responses to leptin are not uniform but are instead specific to the kidney.

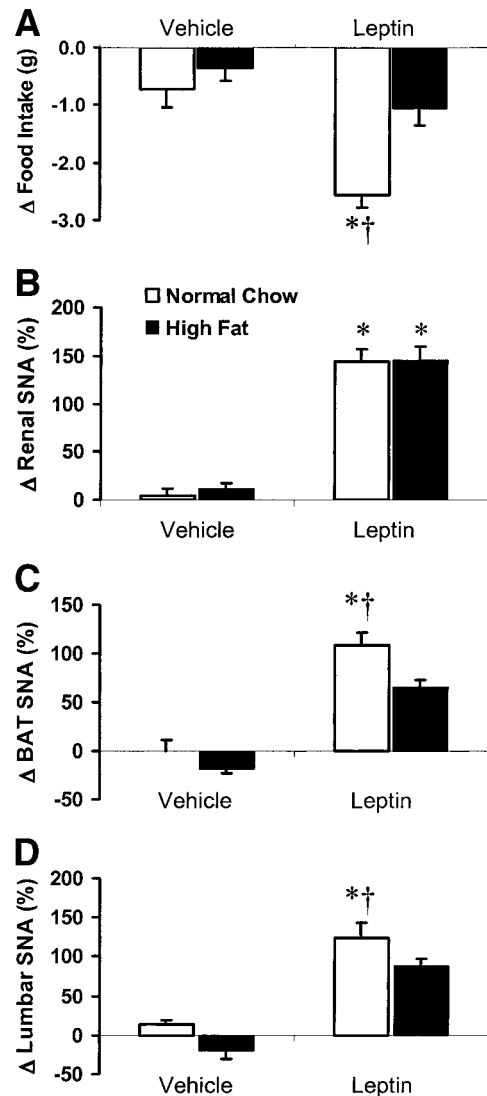


FIG. 5. Change in food intake (A) and renal (B), BAT (C), and lumbar (D) SNA after intracerebroventricular administration of vehicle and leptin (5 μ g) in mice on normal or high-fat diet. Data are means \pm SE of 15–16 animals per group. There was a significant difference between mice on normal or high-fat diet in the effects of leptin on food intake ($P < 0.001$) and BAT ($P = 0.029$) and lumbar ($P = 0.05$) SNA but not renal SNA ($P = 0.917$). * $P < 0.05$ vs. vehicle, † $P < 0.05$ vs. mice on high-fat diet.

In these obese mice, leptin failed to decrease appetite and body weight or to increase lumbar and BAT sympathetic activity. However, leptin was able to induce renal sympathetic activation and increase arterial pressure with similar magnitude in the obese and lean mice. These results demonstrate that selective leptin resistance occurs in dietary obesity as it does in a rare monogenic murine model of obesity, i.e., agouti obese mice. Interestingly, the selectivity in leptin resistance occurs despite the modest increase in body weight and fat mass in mice fed high-fat diet. Moreover, the increases in plasma leptin were also relatively modest (about threefold increase). The response of regional SNA to leptin was differentially altered in the mice fed high-fat diet. Resistance of the thermogenic BAT SNA to leptin is consistent with the attenuated weight-reducing action of leptin. In line with our results here, decreased lumbar sympathetic activation to leptin has

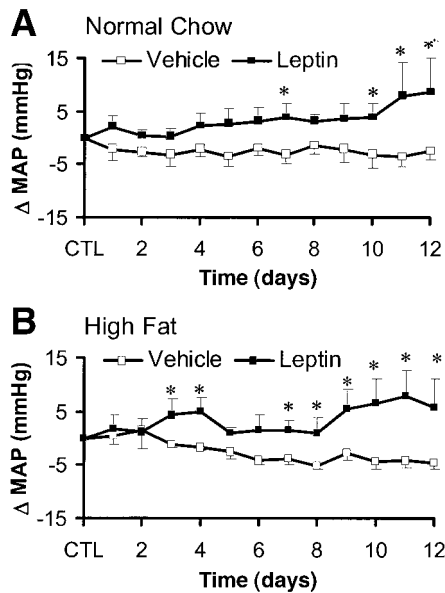


FIG. 6. Change in mean arterial pressure (MAP) induced by 12 days intraperitoneal administration of vehicle and leptin (60 μ g, twice daily) in mice on normal or high-fat diet. Data are means \pm SE of seven to eight animals per group. Leptin raise arterial pressure in mice fed normal diet but also in high-fat-fed mice. * $P < 0.05$ vs. vehicle-treated mice.

been reported in diet-induced obese rat (15). Finally, data from the present study support the concept that there is differential regulation of sympathetic outflow to peripheral tissues.

Preservation of renal sympathetic response to leptin in presence of hyperleptinemia could explain an adverse effect of leptin on blood pressure in obesity, despite resistance to the metabolic actions of leptin. The kidney is known to play a major role in the control of cardiovascular function and blood pressure. The renal effects of increased renal sympathetic outflow include increased renal tubular sodium reabsorption, leading to renal sodium retention; decreased renal blood flow and glomerular filtration rate; renal vasoconstriction; and increased renin release, leading to angiotensin II production (16). These alterations are known to promote increases in arterial pressure. Although the effect of leptin on renal function was not assessed in the present study, we found that leptin produced comparable increases in arterial pressure in obese versus lean mice. The critical role of the sympathetic nervous system in arterial pressure response to leptin has been demonstrated (17). The pivotal role of renal nerves in obesity-induced hypertension has also been shown (18). Altogether, these data indicate that preservation of leptin's ability to stimulate renal sympathetic activity in obesity could explain a role of this hormone in obesity-associated hypertension.

Preserved renal sympathetic activation to leptin in the presence of hyperleptinemia in the obese mice would have been expected to result in higher baseline renal SNA. Using direct nerve recording, we failed to see a difference in renal SNA at baseline between lean and obese mice. However, this method of sympathetic nerve recording is not reliable to quantitative absolute levels of sympathetic activity but is best for measuring responses to short-term stimuli. Interestingly, obese humans exhibit increased

renal norepinephrine spillover (19). Elevated renal sympathetic outflow has also been reported in animal models of dietary obesity (20,21). Eikelis et al. (22) have recently shown a strong correlation between leptin plasma concentration and renal sympathetic activity across a broad range of leptin values in men of widely differing adiposity. This indicates that leptin may be a major cause of renal sympathoactivation associated with obesity in animal models and in humans. In our studies, it is highly likely that leptin stimulated efferent rather than afferent sympathetic activity because distal transection of the renal nerve did not alter the sympathetic response to leptin. In addition, intracerebroventricular administration of leptin, which would not have access to renal afferent nerves, mimics the effect of intravenous leptin on renal sympathetic outflow.

Saturation in the transport of leptin into the central nervous system represents one potential mechanism of selective leptin resistance in obesity (23,24), but as discussed below, we can exclude this explanation. Tanida et al. (25) showed that local injection of leptin in white adipose tissue activate sympathetic activity to the kidney. Therefore, if the renal SNA effects of leptin result from peripheral action, then selective leptin resistance could result from central neural resistance to metabolic effects of leptin and preservation of peripheral sympathetic action of leptin. However, we have shown that the sympatho-excitatory effects of leptin result from an action in the central nervous system. Intracerebroventricular injection of leptin increases SNA in rats and mice ([12,26] and present data), and lesions of the arcuate nucleus in the hypothalamus prevent the sympathetic responses to intravenous administration of leptin (26). In addition, we have shown that selective leptin resistance in obese mice is observed with central neural as well as systemic administration of leptin. These observations exclude a defect in transport of leptin across the blood-brain barrier as an explanation for our results.

The mechanisms of selectivity in leptin resistance are just beginning to be unraveled. Indeed, in obese mice, the inability of leptin to activate intracellular signaling pathways such as the STAT3 proteins appears to be restricted to the arcuate nucleus of the hypothalamus (27). The arcuate nucleus is known to be a major site of leptin action to control food intake and body weight (28,29) but also BAT SNA (26). In contrast, leptin-induced renal sympathetic activation and blood pressure increase are mediated by the ventromedial and dorsomedial hypothalamus (30). Therefore, we speculate that selectivity in leptin resistance may be due to the inability of leptin to activate downstream signaling pathways in the arcuate nucleus but preservation of leptin action in other cardiovascular-related hypothalamic areas. However, future studies are required to test this speculation.

In conclusion, we have shown that in mice, dietary obesity is associated with selective leptin resistance. Systemic and central administration of leptin increased renal SNA but had an attenuated effect on food intake, body weight, and SNA to the hindlimb or BAT. Preservation of renal SNA response to leptin in the obese mice was associated with a preserved arterial pressure response to leptin. Our findings suggest that selectivity in leptin resis-

tance with preservation of renal sympathetic nerve responses to leptin is a potential mechanism for increases in arterial pressure and adverse cardiovascular actions of leptin in obesity.

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REFERENCES

1. Aneja A, El-Atat F, McFarlane SI, Sowers JR: Hypertension and obesity. *Recent Prog Horm Res* 59:169–205, 2004
2. Hall JE: The kidney, hypertension, and obesity. *Hypertension* 41:625–633, 2003
3. Alvarez GE, Beske SD, Ballard TP, Davy KP: Sympathetic neural activation in visceral obesity. *Circulation* 106:2533–2536, 2002
4. Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
5. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI: Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 100:270–278, 1997
6. Dunbar JC, Hu Y, Lu H: Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 46:2040–2043, 1997
7. Shek EW, Brands MW, Hall JE: Chronic leptin infusion increases arterial pressure. *Hypertension* 32:376–377, 1998
8. Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K: Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* 105:1243–1252, 2000
9. Mark AL, Shaffer RA, Correia ML, Morgan DA, Sigmund CD, Haynes WG: Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens* 17:1949–1953, 1999
10. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1:1155–1161, 1995
11. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
12. Correia MLG, Haynes WG, Rahmouni K, Morgan DA, Sivitz WI, Mark AL: Selective leptin resistance in agouti yellow obese mice. *Diabetes* 51:439–442, 2002
13. Rahmouni K, Haynes WG, Morgan DA, Mark AL: Selective resistance to central neural administration of leptin in agouti obese mice. *Hypertension* 39:486–490, 2002

14. Hall JE, Brands MW, Hildebrandt DA, Kuo J, Fitzgerald S: Role of sympathetic nervous system and neuropeptides in obesity hypertension. *Braz J Med Biol Res* 33:605–618, 2000
15. Lu H, Duanmu Z, Houck C, Jen KL, Buisson A, Dunbar JC: Obesity due to high fat diet decreases the sympathetic nervous and cardiovascular responses to intracerebroventricular leptin in rats. *Brain Res Bull* 47:331–335, 1998
16. DiBona GF: The sympathetic nervous system and hypertension: recent developments. *Hypertension* 43:147–150, 2004
17. Carlyle M, Jones OB, Kuo JJ, Hall JE: Chronic cardiovascular and renal actions of leptin: role of adrenergic activity. *Hypertension* 39:496–501, 2002
18. Kassab S, Kato T, Wilkins FC, Chen R, Hall JE, Granger JP: Renal denervation attenuates the sodium retention and hypertension associated with obesity. *Hypertension* 25:893–897, 1995
19. Vaz M, Jennings G, Turner A, Cox H, Lambert G, Esler M: Regional sympathetic nervous activity and oxygen consumption in obese normotensive human subjects. *Circulation* 96:3423–3429, 1997
20. Iwashita S, Tanida M, Terui N, Ootsuka Y, Shu M, Kang D, Suzuki M: Direct measurement of renal sympathetic nervous activity in high-fat diet-related hypertensive rats. *Life Sci* 71:537–546, 2002
21. Barnes MJ, Lapanowski K, Conley A, Rafols JA, Jen KL, Dunbar JC: High fat feeding is associated with increased blood pressure, sympathetic nerve activity and hypothalamic mu opioid receptors. *Brain Res Bull* 61:511–519, 2003
22. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M: Interactions between leptin and the human sympathetic nervous system. *Hypertension* 41:1072–1079, 2003
23. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV: Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348:159–161, 1996
24. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS: Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105:1827–1832, 2000
25. Tanida M, Iwashita S, Ootsuka Y, Terui N, Suzuki M: Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. *Neurosci Lett* 293:107–110, 2000
26. Haynes WG: Interaction between leptin and sympathetic nervous system in hypertension. *Curr Hypertens Res* 2:311–318, 2000
27. Munzberg H, Flier JS, Bjorbaek C: Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145:4880–4889, 2004
28. Dawson R, Pelleymounter MA, Millard WJ, Liu S, Eppler B: Attenuation of leptin-mediated effects by monosodium glutamate-induced arcuate nucleus damage. *Am J Physiol* 273:E202–E206, 1997
29. Tang-Christensen M, Holst JJ, Hartmann B, Vrang N: The arcuate nucleus is pivotal in mediating the anorectic effects of centrally administered leptin. *Neuroreport* 10:1183–1187, 1999
30. Marsh AJ, Fontes MA, Killinger S, Pawlak DB, Polson JW, Dampney RA: Cardiovascular responses evoked by leptin acting on neurons in the ventromedial and dorsomedial hypothalamus. *Hypertension* 42:488–493, 2003.

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