Altered leptin signaling is sufficient, but not required, for hypotension associated with caloric restriction

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Swoap, Steven J. Altered leptin signaling is sufficient, but not required, for hypotension associated with caloric restriction. Am J Physiol Heart Circ Physiol 281: H2473–H2479, 2001.—Caloric restriction of mammals leads to decreases in blood pressure and heart rate. Although relevant clinically, the mechanisms involved, in terms of hormones and signaling pathways invoked, are currently not known. Circumstantial evidence suggests that leptin signaling may be involved with the bradycardia and hypotension associated with caloric restriction. This hypothesis was specifically tested using leptin-deficient mice (ob/ob) or leptin-receptor rats (Koletsky). Ob/ob mice were hypertensive during the light cycle relative to littermate controls (108 ± 2 vs. 100 ± 2 mmHg, respectively). Both ob/ob mice and wild-type mice exhibited hypotension and bradycardia on initiation of a 50% caloric restriction regime, suggesting that the loss of leptin during caloric restriction is not required to explain the cardiovascular effects. Blood pressure in Koletsky rats did not drop in response to caloric restriction during the light cycle, whereas blood pressure in littermate control rats significantly dropped. These data suggest that at least two pathways are involved with cardiovascular effects of caloric restriction: one dependent on leptin signaling and the other independent of the leptin axis.

Thus, in addition to its well-studied role in maintenance of body and fat mass, leptin may be important for the regulation of blood pressure via altering SNS outflow. This leads to the testable hypothesis that CR-induced hypotension is caused by a decrease in circulating leptin, which lowers SNS outflow, which then lowers heart rate and blood pressure.

MATERIALS AND METHODS

Animals. Six-week-old ob/ob female mice and their wild-type littermates were purchased from Jackson Laboratories. Three-month-old female Koletsky rats and their wild-type littermates were purchased from the breeding colony at Vassar College. Animals were maintained at 22°C on a 12:12-h light-dark cycle (dark from 5 PM to 5 AM).

Implantation of blood pressure telemeters. Mice (n = 6 for each group) were anesthetized initially with 5% isofluorane in an oxygen stream and maintained on 1–2% isofluorane in an oxygen stream. The animals were kept on a heating pad (38°C) throughout the surgery. Blood pressure transducers (model PAC20; Data Sciences International) were implanted in the left common carotid artery of the mouse as described previously (3), with one exception. Instead of tunneling and placing the radiotelemeter unit on the contralateral side, we sutured the unit into the body wall of the peritoneal cavity. For implantation into the rats (n = 5 for each group), the animals were anesthetized as above, and the abdominal aorta rostral to the bifurcation into the iliac arteries was dissected away from the vena cava. A 2-0 suture was placed under the aorta and pulled taut to prevent blood flow. A 21-g needle bent to 90° was used to puncture the aorta rostral to the bifurcation. The catheter of the blood pressure telemeter (model PAC20; Data Sciences International) were implanted in the left common carotid artery of the mouse as described previously (3), with one exception. Instead of tunneling and placing the radiotelemeter unit on the contralateral side, we sutured the unit into the body wall of the peritoneal cavity. For implantation into the rats (n = 5 for each group), the animals were anesthetized as above, and the abdominal aorta rostral to the bifurcation was dissected away from the vena cava. A 2-0 suture was placed under the aorta and pulled taut to prevent blood flow. A 21-g needle bent to 90° was used to puncture the aorta rostral to the bifurcation. The catheter of the blood pressure telemeter (model PAC20; Data Sciences International) were implanted in the left common carotid artery of the mouse as described previously (3), with one exception. Instead of tunneling and placing the radiotelemeter unit on the contralateral side, we sutured the unit into the body wall.

Implantation of temperature telemeters. Core temperature in ob/ob and wild-type mice (n = 5 for each) was measured by abdominal cavity implantation of temperature telemeters (model TAF20, Data Sciences International). Animals were anesthetized as above, and a 1-cm incision was made along the midline. After insertion of the temperature implant into the abdominal cavity, the incision was closed with VetBond and a cellulose fiber patch. The transmitter was sutured into the body wall.

Caloric restriction. Ten days after the implantation surgery, animals started a diet consisting of the following: 45% carbohydrate (cornstarch), 35% fat (24% corn oil and 11% lard), and 20% protein (casein), supplemented with vitamins, minerals, and fiber (cellulose). Animals remained on this diet for hypotension associated with caloric restriction.

A RELATIONSHIP BETWEEN DIET and blood pressure has been recognized for many years (15). Prolonged caloric restriction (CR) significantly lowers blood pressure in hypertensive humans, normotensive rats, and in many models of hypertension in rats (8, 14, 25, 27, 30). The autonomic nervous system appears to mediate this hypotensive effect and bradycardia of CR. Sympathetic nervous system (SNS) outflow is reduced during a CR regimen, and that reduction in SNS activity correlates with a decrease in blood pressure (8, 15, 23, 29).

Much evidence links the hormone leptin, the protein product of the ob gene (31), with increased sympathetic nervous system outflow. Intracerebroventricular infusion of leptin leads to sympathetic activation of brown adipose tissue, kidney, adrenal, and lumbar regions (6, 12). Intracerebroventricular infusion of leptin or chronic intravenous leptin infusion also increases mean arterial blood pressure and heart rate (6, 24).

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for 10 days. During this time, total caloric intake was measured. These wild-type mice ate \( \sim 16 \text{ kcal/day} \), whereas the \( \text{ob/ob} \) mice ate \( \sim 24 \text{ kcal/day} \). The control rats ate \( \sim 130 \text{ kcal/day} \) and the Koletsky rats ate \( \sim 180 \text{ kcal/day} \). After 10 days, animals were fed 50% of normal caloric intake supplemented with vitamins and minerals to match the intake of the pre-CR diet. Because the food used during the CR period was supplemented with vitamins and minerals, intake of all vitamins and minerals, including sodium, was equivalent to that taken in during ad libitum feeding. The mice were calorically restricted for 7 days. The rats were calorically restricted for 28 days. Animals were always fed, both during ad libitum feeding and CR feeding, at the onset of the dark cycle, 5 PM.

**Cardiovascular data collection.** Data from the telemeters were recorded at 500 Hz. Between 5 PM and 4 PM on the following day, 5-s bouts of data were obtained every 2 min. From the pressure waveform analysis, the following cardiovascular parameters were obtained: heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, and pulse pressure. Activity of the animals was also monitored and reported every 2 min. Between 4 PM and 5 PM, data were not taken while the animals were weighed and fed. The dark cycle cardiovascular parameters or temperature were averaged from data collected between 7 PM and 4 AM to avoid meal-related changes in hemodynamics. The light-cycle parameters were averaged from data collected between 7 AM and 4 PM, although no difference was found when data were averaged from data collected between 5 AM and 4 PM.

**Northern blotting.** RNA was isolated from mouse ventricles as described previously (25). The blot was probed with an oligonucleotide specific for the mouse \( \beta \)-myosin heavy chain (\( \beta \)-MHC) mRNA as described previously (25). The sequence of the oligonucleotide was 5’-CCACCTAAAGGGCTGTTG-CAAAGGC-3’.

**Statistics.** Data for the variables studied are reported as means \( \pm \) SE. Statistically significant differences were determined by using Dunn’s test after analysis of variance. The \( P < 0.05 \) level of confidence was accepted for statistical significance.

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**Fig. 1.** Mean blood pressure, heart rate, and activity levels for wild-type and leptin-deficient (\( \text{ob/ob} \)) mice. The left common carotid artery of \( \text{ob/ob} \) mice (\( n = 6 \)) and wild-type mice (\( n = 6 \)) was implanted with a blood pressure telemeter. After recovery from the surgery (10 days), mean blood pressure, heart rate, and activity levels were monitored in these animals for 3 days while the animals ate ad libitum (Ad lib). Data were collected for 5 s every 2 min. Data were averaged for the light cycle (7 AM–4 PM) and the dark cycle (7 PM–5 AM). Animal diets were then restricted to 50% of the calories normally eaten (CR), with vitamin and mineral supplementation to 100%. Data were collected for the next 2 days and averaged for the light cycle and dark cycle. \(* P < 0.05 \) vs. wild-type ad libitum. \( ** P < 0.05 \) vs. \( \text{ob/ob} \) ad libitum. Note that free-eating \( \text{ob/ob} \) mice were hypertensive relative to free-eating wild-type mice during the light cycle. Note also that the mean blood pressure and heart rate (in beats/min) of both \( \text{ob/ob} \) mice and wild-type mice significantly dropped in both the dark and light cycles.
RESULTS

Ob/ob mice versus wild-type littermates. Ob/ob mice were found to be hypertensive relative to wild-type littermates during the light cycle (Fig. 1). During this period, ob/ob mice had similar levels of activity as the wild-type littermates ($P = 0.209$). However, during the dark cycle, ob/ob mice were much less active than wild-type littermates, yet had no difference in mean blood pressure (Fig. 1). The increase in blood pressure in wild-type animals in the dark cycle correlated with the large increase in activity levels of these animals. In both the dark and light cycles, heart rate was significantly lower in the ob/ob mice (Fig. 1). On examination of the data over 24 h (i.e., the dark and light cycles combined), ob/ob mice were normotensive compared with wild-type controls ($111 \pm 1$ vs. $109 \pm 2$ mmHg, respectively) and had lower heart rates than wild-type controls ($560 \pm 9$ vs. $613 \pm 13$ beats/min, respectively).

Ad libitum versus CR in mice. On 50% CR of ob/ob and wild-type mice, both strains of mice exhibited

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Fig. 2. Pressure and temperature changes in an ob/ob mouse during prolonged CR. A: typical blood pressure tracing of an ob/ob mouse before (ad libitum) and after 50% CR over a 5-s period. These data were obtained from the same animal (see B) at 4:48 AM before and after 7 days of CR. B: heart rate, mean blood pressure, and activity levels of this ob/ob mouse before (ad libitum) and after 50% CR over a 24-h period. The 24-h time period shown for the animal during CR is 7 days after the initiation of CR. The calorically restricted animal received 12 kcal of food at the onset of the dark cycle (5 PM). Heart rate, blood pressure, and activity levels were monitored every 2 min for 5 s. Heart rate fell to a minimum of 132 beats/min. Mean blood pressure fell to a minimum of 63 mmHg in this mouse. C: temperature and activity levels of a different ob/ob mouse before (ad libitum) and after 50% CR over a 24-h period. The 24-h time period shown for the animal during CR is 7 days after the initiation of CR. The calorically restricted animal received 12 kcal of food at the onset of the dark cycle (5 PM). Temperature and activity levels were monitored every 2 min for 5 s. Room temperature (RT) during the 24-h period is shown.
significant drops in mean blood pressure and heart rate (Fig. 1). These changes occurred within two days of initiation of CR, and were manifest in both the dark cycle and light cycle. Heart rate also dropped in both strains of mice in response to CR. These changes in cardiovascular parameters occurred in the absence of changes in activity patterns (Fig. 1). Over 24 h, heart rates and mean blood pressure significantly dropped relative to ad libitum feeding in both the ob/ob animals (96 ± 5 mmHg and 417 ± 26 beats/min) and in wild-type control animals (103 ± 2 mmHg and 537 ± 13 beats/min).

Long-term response of cardiovascular parameters in response to CR of mice. After 3–4 days of CR, both wild-type (not shown) and ob/ob animals (Fig. 2, A and B), exhibited very large changes in pressure tracings indicative of animals entering into torpor. To determine whether this was the case, ob/ob and wild-type animals were implanted with temperature telemeters. After 4 days of CR, the core temperature of all mice tested dropped to −2–5°C above ambient temperature for periods of time ranging from 4 to 9 h on a daily basis (Fig. 2C). This indicates that the dramatic changes in cardiovascular parameters observed after 3–4 days of CR (Fig. 2, A and B) was the cardiovascular response to torpor. Interestingly, before the onset of CR, ob/ob core temperatures were significantly lower than wild-type core temperatures: light cycle, 35.7 ± 0.2 vs. 36.7 ± 0.1, respectively, and dark cycle, 36.1 ± 0.1 vs. 37.8 ± 0.1, respectively.

β-MHC mRNA expression. One hallmark of CR in the rat is the induction of β-MHC mRNA and protein in the heart (25). To assess whether the same is observed in mice, RNA from the cardiac ventricles was probed for the β-MHC mRNA (Fig. 3). As is clearly shown, CR induces β-MHC mRNA expression in both wild-type and ob/ob cardiac tissue.

Koletsky rats versus control rats. To further examine leptin and leptin signaling as a potential mediator of the hypotensive effect of CR, cardiovascular parameters of spontaneously hypertensive obese rats (Koletsy) were measured. Koletsy rats, which because of a mutation in the leptin receptor gene express no functional forms of the leptin receptor (26), were hypertensive relative to control animals, as has been seen previously (9, 13), during both the light and dark cycles (Fig. 4). These animals also exhibited lower heart rates than control animals. For 24-h measurements, Koletsy rats were hypertensive relative to littermate controls (102 ± 2 vs. 94 ± 1 mmHg, respectively) and exhibited a lower heart rate than littermate controls (369 ± 7 vs. 399 ± 3 beats/min, respectively).

Ad libitum versus CR in Koletsky rats. After 4 wk of 50% CR, mean blood pressure in control rats dropped during both the light and dark cycles (Fig. 4). However, the mean blood pressure in calorically restricted Koletsy rats did not differ from the ad libitum values in the light cycle (P = 0.659). There was a slight, but significant, drop in the mean blood pressure of Koletsy rats during the dark cycle. Especially relevant to these blood pressure findings are the activity levels in these animals. Unlike the activity of the wild-type mice, which did not change on CR within the first two days, Koletsy rats became more active during the light cycle (Fig. 3). When examined over the 24-h period, control animals’ mean blood pressure dropped significantly to 84 ± 3 mmHg, whereas Koletsky mean blood pressure was not significantly different from free-eating mean blood pressure (99 ± 3 mmHg). Heart rates in all rats dropped on CR. Light- and dark-cycle heart rates are shown in Fig. 4. Twenty-four-hour heart rates during CR were as follows: Koletsy, 307 ± 7 beats/min, and controls, 311 ± 5 beats/min.

DISCUSSION

It is well recognized that obesity in humans is associated with high blood pressure (15). Recent evidence suggests that the elevated levels of leptin in obese subjects may be responsible for the observed hypertension. For example, central infusion of leptin, or chronic peripheral infusion of leptin, leads to an increase sympathetic nerve traffic and increases in blood pressure (4, 6, 12, 18, 24), although this is not observed universally (5). Furthermore, mice that overexpress leptin, so-called “transgenic skinny” mice, have elevated tail cuff systolic blood pressure (2). Adipose tissue, circulating leptin, and blood pressure all fall during a calorically restricted diet. Thus it has been proposed that the hypotension and bradycardia associated with a calorically restricted diet is caused by the decrease in circulating leptin levels (1). In fact, Overton et al. (21) recently demonstrated that central infusion of leptin during fasting in rats can prevent bradycardia associated with acute fasting.

Therefore, ob/ob mice (missing leptin) and obese spontaneously hypertensive rats (Koletsy), which harbor a mutation in the leptin receptor that render it nonfunctional (26), were used herein to test the specific hypothesis that the hypotension and bradycardia that occur during CR are mediated through the leptin signaling pathway. This hypothesis generates two predictions of cardiovascular parameters in ob/ob mice. First, in the absence of this hormone (e.g., ob/ob mice), animals should be hypertensive under normal conditions.

![Image](http://ajpheart.physiology.org/)
Second, because there is no drop in leptin in \textit{ob/ob} mice during CR, there should be no change in mean blood pressure during CR of these mice. Data obtained herein do not support either of these predictions. It was found that the mean blood pressure of the \textit{ob/ob} animals was significantly elevated relative to that of their wild-type littermate controls in the light cycle (Fig. 1). These data differ from previous investigations (2, 17), which found hypotension in \textit{ob/ob} mice. The discrepancies in blood pressure in \textit{ob/ob} mice may be explained by methodologies used to measure blood pressure. Others have used either a tail cuff (2) or a tethered carotid line (17). With the use of radiotelemetry, blood pressure was measured 24 h/day, which obviously includes all activities. One activity, feeding, may be especially relevant because the \textit{ob/ob} animals eat throughout both the night and day, potentially leading to the hypertension observed during the day. This difference in blood pressure between \textit{ob/ob} mice and littermates is obscured during the dark cycle probably because of the large increase in activity in wild-type mice in the dark cycle (Fig. 1), and the lack of increase in activity in the \textit{ob/ob} mice.

The second prediction, that the blood pressure of \textit{ob/ob} mice should not drop on CR, also did not become manifest. After the first 2 days, blood pressure dropped significantly in both \textit{ob/ob} mice and littermates (Fig. 1). This suggests that the signal for the drop in blood pressure during CR is not the change in circulating leptin during CR.

However, data obtained herein suggests that the leptin signaling pathway can be involved in the cardiovascular effects of CR. By using obese spontaneously hypertensive rats (Koletsky), we show herein that a
functional leptin receptor is required for the hypotensive effects of CR, at least during the light cycle. The mean blood pressure of the obese spontaneously hypertensive rats did not drop with CR during the light cycle, as did the pressure of littermate controls (Fig. 4) and Sprague-Dawley rats (data not shown). The lack in drop of the blood pressures in the obese spontaneously hypertensive rats confirms previous tail-cuff systolic blood pressure measurements of these animals (9, 13). In addition, others have shown that leptin signaling may be involved with the cardiovascular effects of energy deprivation. For example, it is well known that neuropeptide Y (NPY) is a major mediator of leptin signaling, and its expression is diminished on leptin administration. Intracerebral injection of NPY lowers plasma norepinephrine, decreases SNS activity to brown adipose tissue, lowers the heart rate, and lowers blood pressure (7, 11, 19). Thus increased activation of NPYergic activity in the hypothalamus could contribute to sympathoinhibitory and hypotensive effects of reduced caloric intake. In fact, the central administration of a NPY antagonist-o-NPY (27–36) blunts the fall in blood pressure associated with CR (27).

Thus it is apparent that there exists a complex relationship between circulating leptin, leptin signaling, CR, and blood pressure. The data obtained from the obese spontaneously hypertensive rats (Fig. 4 and Refs. 9 and 13), as well as NPY antagonists (27), suggest that altered leptin signaling is sufficient to mediate the hypotension and bradycardia associated with energy deprivation. However, the data obtained from the ob/ob mice herein, and data from calorically restricted obese Zucker rats (22), which have reduced responsiveness to leptin, suggest that leptin is not required for the cardiovascular effects of CR. That is, there must be additional signals not related to leptin that are invoked on CR and mediate the hypotension associated with CR.

Interestingly, mice fed 50% of normal caloric intake for >3 days entered into a daily torpor. Torpor is characterized by large changes in core body temperature and metabolic rate, substantially lowering daily energy expenditure. This highly conserved adaptation to cold ambient temperatures and/or low food availability is found in many small mammals. Both wild-type and ob/ob mice entered torpor, suggesting that the drop in circulating leptin is not the signal that sends the animal into torpor, as has been suggested previously (10). The cardiovascular parameters observed during torpor are quite striking. Systolic (not shown), mean (Fig. 2), and diastolic (not shown) blood pressures all drop ~35% from free eating control pressures. A typical diastolic blood pressure during torpor was 56 mmHg. Heart rates drop to one-fifth of free eating controls, to ~130 beats/min in these mice. Similar changes in cardiovascular parameters have been observed in hibernating 13-lined ground squirrels (16). Rats, which have a substantially lower surface area-to-body weight ratio than mice and a substantially greater ability for absolute energy storage, do not enter torpor, even in the presence of low energy supply at 22°C.

In conclusion, it is shown here using genetic models of altered leptin signaling that at least two pathways are invoked that mediate the hypotension associated with CR. First, leptin signaling certainly seems to play a role in modulating blood pressure during CR, as evidenced by animals missing the functional leptin receptor (Koletsky) as well as data from others (28). Second, using ob/ob mice, we may have detected a second pathway to mediate the cardiovascular effects of CR, but not associated with leptin. It may be that in the absence of leptin action in the ob/ob mice, other pathways are activated that are not normally brought into play with CR when the leptin signaling pathway is functional.

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REFERENCES


