Obesity-Related Hypertension and the Role of Insulin and Leptin in High-Fat–Fed Rabbits

Kyungjoon Lim, Sandra L. Burke, Geoffrey A. Head

Abstract—Feeding a high-fat diet (HFD) to rabbits results in increased blood pressure and renal sympathetic nerve activity (RSNA) and marked increases in plasma leptin and insulin. We determined the contribution of insulin and leptin signaling in the central nervous system to the increased blood pressure and RSNA during a HFD using specific antagonists. New Zealand White rabbits were implanted with an intracerebroventricular (ICV) catheter and RSNA electrode and placed on a normal or 13.5% HFD for 1 or 3 weeks. Blood pressure, heart rate, and RSNA were recorded before and for 90 minutes after ICV administration of a leptin antagonist (100 µg), insulin antagonist (0.5 U), or vehicle (50 µL) on separate days. Rabbits had higher blood pressure (+8%, +17%) and RSNA (+55%, +71%), at 1 and 3 weeks, respectively, of HFD compared with controls (n=7–11). ICV leptin antagonist reduced blood pressure by 9% and RSNA by 17% (P<0.001) after 3 weeks of HFD but had no effect at week 1. ICV administration of the insulin antagonist reduced blood pressure by ≈5% at both times (P<0.05) but there was no effect on RSNA. Leptin and insulin antagonist doses were confirmed to effectively block the pressor responses to ICV leptin and insulin, respectively. The elevation of blood pressure and RSNA induced by a HFD is predominantly mediated by central actions of leptin. Central actions of insulin contribute a smaller proportion of the hypertension but independently of RSNA. (Hypertension. 2013;61:628-634.)

Key Words: high-fat diet ■ hypertension ■ insulin ■ leptin ■ obesity ■ renal sympathetic nerve activity

Obesity is now a large-scale global epidemic that develops from a complex interaction between genotype and environment, including social, behavioral, cultural, physiological, and metabolic factors. The consequences of being overweight or obese include increased incidence of hypertension, hyperlipidemia and hyperinsulinemia, insulin resistance, and diabetes mellitus. Clinical evidence suggests that sympathetic activation participates not only in the initial elevation in blood pressure but also in maintaining the hypertension. Obesity is closely linked to increased sympathetic nerve activity (SNA) to the kidneys and skeletal muscle vasculature and is linked to the accumulation of body fat.

Our focus has been on the mechanism underlying the increase in blood pressure and, in particular, the role of the sympathetic nervous system in the development of the hypertensive state. We have demonstrated that 3 weeks of high-fat diet (HFD) feeding leads to increased mean arterial pressure (MAP), heart rate (HR), and renal SNA (RSNA) in rabbits. Importantly, we have shown that ganglion blockade completely abolishes the increase in blood pressure suggesting that this model of obesity hypertension is neurogenic. We suggested that the mechanism of the hypertension involved sympathetic activation and increased responsiveness to central sympathoexcitatory effects of leptin owing to increased plasma leptin arising from visceral fat accumulation. Leptin is an adipokine that plays an important role in regulating energy intake and energy expenditure, including appetite and metabolism, and acts as a key peripheral hormone in distinct neurons in the hypothalamus. Leptin is secreted primarily by adipocytes and is present in serum in direct proportion to the amount of adipose tissue. Chronic leptin infusion has been shown to increase HR and blood pressure in animal models via stimulation of the sympathetic nervous system. In addition to the rise in leptin, we also observed a marked increase in plasma insulin in rabbits fed a HFD suggesting that insulin may also contribute to the hypertension and increased RSNA in rabbits particularly early in the onset of the HFD before significant accumulation of visceral fat. Thus, both leptin and insulin may contribute to the hypertension, and with the development of specific antagonists it is now possible to determine their relative roles. In the present study, we administered either the insulin receptor antagonist or the leptin receptor antagonist intracerebroventriculatry to conscious rabbits at 1 or 3 weeks after the onset of a HFD.

Materials and Methods
Twenty-seven male New Zealand White rabbits (initial body weight, 2.6–3.1 kg) were housed under controlled light (lights on 6:00

Received November 25, 2012; first decision December 20, 2012; revision accepted December 21, 2012.
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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.111.00705/-/DC1.
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AM to 6:00 PM) and temperature (22±2°C) conditions. Water was available ad libitum. The experiments were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for Scientific Use of Animals.15

Experimental Procedures and Protocol

Rabbits were divided into 3 groups and meal fed either a normal diet (ND, 140 g/d; 2.63 kcal/g) or a HFD (190 g/d; 3.34 kcal/g; 13.3% fat) for 1 or 3 weeks.15 Rabbits underwent 2 preliminary surgical operations under isoflurane anesthesia (3%–4% in 1 L/min oxygen) after induction with propofol (10 mg/kg IV, Fresenius Kabi, Pymble, NSW, Australia). Carprofen (3 mg/kg SC, Pfizer, North Ryde, NSW, Australia) was given 30 minutes beforehand and 24 hours later for analgesia. An intracerebroventricular (ICV) catheter (Plastics One, Roanoke, VA) was first implanted into the lateral ventricle (coordinates from bregma; 3-mm lateral and 4-mm ventral), 1 week before starting allocated diets, as described previously.15 Secondly, a recording electrode was implanted on the left renal nerve for the recording of RSNA14 1 week before the first experiment.

Measurement of Cardiovascular Variables and RSNA

Experiments were conducted in conscious rabbits held in a standard single rabbit holding box. MAP and HR were measured from the central ear artery catheterized transcutaneously.15 MAP, HR, derived from the pressure pulse, and RSNA were digitized at 500 Hz using an analog-to-digital data acquisition card (National Instruments 6024E, Austin, TX) and averaged over 2 seconds. RSNA was measured in microvolts (μV) normalized to the maximum value that was elicited by the nasopharyngeal reflex (=100 nU), evoked by exposing the rabbit to cigarette smoke.15

Dose–Response Curve and Antagonist Experiments

In separate rabbits, an initial dose–response experiment was conducted using insulin and leptin in rabbits fed a HFD for 1 week (n=5) or 3 weeks (n=4), respectively. After 1 hour recovery, MAP and HR were recorded for 30 minutes followed by an initial 50 μL ICV injection of the vehicle (Ringer’s solution, Baxter, Old Toongabbie, NSW, Australia). Doses of leptin (recombinant murine leptin 450-31, Pepro Tech, Inc, Rocky Hill, NJ) or insulin (Actrapid, Novo Nordisk, Baulkham Hills, NSW, Australia) were then given ICV at 30-minute intervals. A time control included 4 injections of 50-μL Ringer’s solution (n=6). Each drug was dissolved in 50 μL of vehicle and was given on a separate day with 1 day recovery between each.

In the main experiments, we used a dose of antagonist equivalent to the maximum effective dose of each agonist. To determine whether these doses of antagonist were effective, rabbits, fed a HFD for 1 week, were given 50 μL Ringer’s solution ICV or insulin antagonist (0.5 U ICV, S961, Novo Nordisk, Baulkham Hills, NSW, Australia; n=3) followed 30 minutes later by the same dose of insulin. Another group of rabbits (n=4) was fed a HFD for 3 weeks and given Ringer’s or leptin antagonist (100 μg ICV, LAN3, mutant mouse leptin L39A/D40A/F41A, Protein Laboratories Rehovot Ltd, Rehovot, Israel) followed 30 minutes later by the same dose of leptin.

Main Experiments

Each rabbit in this cohort received 3 drugs (Ringer’s, insulin receptor antagonist, and leptin receptor antagonist) in random order on separate days with 1 day between. Doses were determined from the dose–response experiments and given in a volume of 50 μL. After a 30-minute baseline recording of MAP, HR, and RSNA, all rabbits were administered ICV vehicle (50 μL). Thirty minutes later, 100 μg of the leptin receptor antagonist (n=6–9) or 0.5 U insulin receptor antagonist (n=6–10) or 50 μL vehicle (n=5–9) was administered. MAP, HR, and RSNA were recorded for 90 minutes after each injection. At the end of the experiment rabbits were killed by an anesthetic overdose (160 mg/kg IV, sodium pentobarbitone, Virbac, Milperra, NSW, Australia) and tissues removed (see online-only Data Supplement).

Data Analysis

Values are expressed as mean±SEM and mean±SE of the difference. Data were analyzed by multifactor repeated measures ANOVA that allowed for between and within-animal contrasts. The main effects were diet, antagonist, and week with nonorthogonal contrasts, including (1) comparison between normal and HFD 1 week group, (2) between normal and HFD 3-week group, (3) HFD 1- and 3-week groups, and (4) between insulin and leptin at weeks 1 and 3. For dose–response curves, the between-doses sums of squares were partitioned to determine linear trend. In each case a combined residual term was used as described previously.16 Type 1 error was controlled using Bonferroni and Greenhouse–Geisser corrections. A P value of <0.05 was considered significant.

Results

Effect of HFD After 1 and 3 Weeks

Mean body weight before the rabbits were placed on their respective diets was 2686±45 g. Rabbits fed a HFD gained 236±91 and 407±113 g after 1 and 3 weeks, respectively (P=0.04 and 0.01, respectively). Body weights of rabbits on a ND for 1 or 3 weeks were similar and hence data from the 2 groups were pooled. The average increase in pooled body weight of ND rabbits was 114±44 g (P=0.03). Caloric intake after 1 week of HFD was 71% greater than in rabbits on a ND (n=6; P group<0.001) but after 3 weeks of HFD, caloric intake had declined markedly and was only 32% greater (n=6; P group<0.001; Table). In rabbits fed the HFD for 1 week, both MAP (+8%) and RSNA (+55%) were greater than in ND rabbits (P group<0.05; Table). After another 2 weeks of diet, MAP

Table. Resting Values at First Experiment

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Normal</th>
<th>HFD Week 1</th>
<th>HFD Week 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>71±2</td>
<td>77±2</td>
<td>83±1</td>
<td>0.015</td>
<td>0.000</td>
<td>0.014</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>185±4</td>
<td>217±5</td>
<td>210±5</td>
<td>0.000</td>
<td>0.000</td>
<td>0.188</td>
</tr>
<tr>
<td>RSNA, nU</td>
<td>6.7±0.6</td>
<td>10.5±1.5</td>
<td>11.5±2.0</td>
<td>0.024</td>
<td>0.014</td>
<td>0.363</td>
</tr>
<tr>
<td>RSNA, μV</td>
<td>26.0±3.4</td>
<td>30.3±4.4</td>
<td>24.7±3.6</td>
<td>0.258</td>
<td>0.499</td>
<td>0.235</td>
</tr>
<tr>
<td>Nasopharyngeal, μV</td>
<td>434±104</td>
<td>328±61</td>
<td>222±25</td>
<td>0.375</td>
<td>0.131</td>
<td>0.457</td>
</tr>
<tr>
<td>Calorie intake, kcal/day</td>
<td>368±1</td>
<td>631±2</td>
<td>485±14</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are mean±SEM in rabbits on a normal diet or a high-fat diet for 1 and 3 weeks. P1 is the probability for comparison between normal and HFD 1-week groups, P2 the probability for comparison between normal and HFD 3-week groups, and P3 is the probability for comparison of HFD 1- and 3-week groups and shown in italics if P<0.05. HFD indicates high-fat diet; HR, heart rate; MAP, mean arterial pressure; and RSNA, renal sympathetic nerve activity.
and RSNA increased further to be 18% and 71% greater, respectively, than in ND rabbits (Table). By contrast, HR was 17% to 19% greater than in ND rabbits after both 1 and 3 weeks of HFD (Table).

Dose–Response Curves for Administration of Insulin and Leptin

To determine the maximal dose of insulin, increasing doses of insulin were administered ICV (0.0005–0.5 U), which produced dose-dependent increases in MAP in rabbits fed a HFD for 1 week and a trend for MAP to increase after 3 weeks on the HFD (P<0.06; Figure 1). The increases in MAP at the highest dose were similar in the 2 groups (5.8±3.8 and 4.3±0.8 mm Hg for 1 and 3 weeks of HFD, respectively; Figure 1). HR also increased markedly in response to ICV insulin in both HFD groups but the tachycardia was greater after 3 weeks compared with 1 week (16±4 versus 9±3 bpm, respectively; P<0.05; Figure 1). Increasing doses of leptin (5–100 µg) did not change MAP in rabbits on 1 week of HFD but there was a marked increase in MAP after 3 weeks (6.1±1.5 mm Hg at the highest dose of 100 µg; P<0.01). Leptin did not alter HR after either 1 or 3 weeks of HFD (Figure 1). Thus, the doses of insulin and leptin, which produced the maximum increases in MAP, were 0.5 U and 100 µg, respectively. We determined whether the antagonists administered at these doses were effective in blocking the actions of insulin and leptin. In rabbits fed a HFD for 1 week and pretreated with the insulin antagonist, a single dose of insulin (0.5 U) did not alter MAP, compared with an increase in MAP of 5.9±1.2 mm Hg when pretreated with Ringer’s solution (P<0.01; Figure 1). In rabbits fed a HFD for 3 weeks and pretreated with the leptin antagonist, a single dose of leptin (100 µg) lowered MAP by 4.1±0.9 mm Hg compared with an increase of 4.7±0.6 mm Hg when vehicle was administered before leptin (P<0.001; Figure 1). Consecutive 50-µL doses of Ringer’s solution did alter MAP or HR (Figure 1).

Effect of Insulin Receptor Antagonist

ICV injection of the insulin receptor antagonist (0.5 U) lowered MAP similarly in both HFD groups (−3.9±0.5 and −3.4±0.8 mm Hg for weeks 1 and 3, respectively, averaged over 90 minutes) but not in the rabbits on a ND (P<0.05; Figure 2). The onset of the hypotension occurred within 15 minutes of administration and lasted for the duration of 90-minute recording period. There was a small but significant decrease in HR after insulin antagonist administration in the rabbits fed a HFD for 3 weeks (−5±2 bpm over 90 minutes) compared with ND-fed rabbits in which there was no change in HR (P<0.001; Figure 2). RSNA was not altered by the insulin antagonist in any group (Figure 2). Each rabbit received an initial ICV injection of Ringer’s solution before the antagonist, which had no effect on MAP, HR, or RSNA over a 30-min period compared with baseline control values (Figure 2).

Effect of Leptin Receptor Antagonist

ICV administration of the leptin antagonist (100 µg) produced a marked decrease in MAP of 7.5±1.0 mm Hg (average over 90 minutes) in rabbits fed a HFD for 3 weeks but had no effect on MAP after 1 week of HFD or on rabbits fed a ND (P<0.001; Figure 3). The fall in MAP occurred within 15 minutes of injection and by 30 minutes had completely abolished the difference in MAP between groups. MAP was still attenuated 90 minutes later. The hypotension after administration of leptin antagonist in rabbits fed a HFD for 3 weeks was greater than the hypotension observed after administration of insulin antagonist (F=14.0; P<0.001) and was accompanied by a reduction in RSNA of 3.4±1.4 nA (P<0.001), which did not occur immediately but followed a slower timecourse over 90 minutes from administration (Figure 3). Leptin antagonist administration had no effect on RSNA in either the 1-week HFD rabbits or the ND rabbits. The only measureable effect on HR occurred in the 1-week HFD group in which there was a small but significant tachycardia (3±1 bpm; Figure 3). The initial ICV injection of Ringer’s solution had no effect on MAP, HR, or RSNA (Figure 3).

Figure 1. Line graphs: changes (Δ) from baseline in mean arterial pressure (MAP) and heart rate (HR) in response to insulin. Ringer’s solution (top) or leptin (bottom) in rabbits fed a high-fat diet (HFD) for 1 week (gray circles) or 3 weeks (black circles). Increasing intraventricular doses of insulin or leptin or of Ringer’s solution were given at 30-minute intervals. Values are mean±SE of the difference indicating variance between animals. *P<0.05, ***P<0.001 for comparison between weeks 1 and 3. **P<0.01, and ***P<0.001 for linear trend. Bar graphs: changes in MAP and HR in response to a single intraventricular dose of insulin (0.5 U, top) in rabbits on a HFD for 1 week or leptin (100 µg, bottom) in rabbits on a HFD for 3 weeks. The agonists were administered 30 minutes after Ringer’s solution (gray or black bars) or the appropriate antagonist (hatched bars). Values are mean±SE of the difference. **P<0.01 for comparison of effect of Ringers vs antagonist.
Effect of Vehicle
All rabbits received ICV administration of the vehicle Ringer’s solution on a separate day instead of the antagonist. MAP, HR, or RSNA levels over 90 minutes were similar to control values (Figure 4).

Adipose Tissue Weight and Body Composition
Adipose tissue weight (removed manually from perirenal, omental, and epididymal areas and between the scapulae) in rabbits fed a HFD for 1 and 3 weeks was markedly greater (+207% and +241%, respectively) than in rabbits on a ND. Similarly, when expressed relative to body weight, adipose tissue weight was also increased in both HFD groups (+178% and +221%) compared with ND rabbits. There was no difference in absolute or relative adipose tissue weight between rabbits on a HFD for 1 or 3 weeks. However, dual-energy x-ray absorptiometry scanning showed that total % of all body fat was greater in 1-week HFD rabbits (+104%; P=0.002) and further increased in 3-week HFD rabbits (+179%; P<0.001) when compared with ND rabbits (Table). Furthermore, 3-week HFD rabbits exhibited 36% higher total % fat than 1-week HFD rabbits (P=0.02; Table S1 in the supplementary information).

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online-only Data Supplement). There were no differences in relative lean muscle mass or in bone mineral content between groups (Table S1 in the online-only Data Supplement).

**Discussion**

The major findings of the current study were that short-term feeding of a HFD to rabbits induced hypertension at 1 week that was inhibited by ICV injection of an insulin antagonist but not by a leptin receptor antagonist. However, by 3 weeks the hypertension and the activation of the sympathetic nervous system were markedly reduced by central injection of a specific leptin antagonist. At this time the insulin receptor antagonist reduced blood pressure slightly but had no effect on RSNA. We have previously shown the strong relationship between the elevation of cardiovascular variables and circulating hormones, particularly between HFD-induced increases in RSNA and plasma leptin. The present study provides our first evidence of a clear causal relationship with central leptin signaling once leptin levels are increased (current study) and persistence of the hypertension and high RSNA levels in the presence of normal levels of insulin and leptin may be attributable to central actions of other adipokines or elevated plasma fatty acids. However, this now seems unlikely with the current findings where insulin seems mainly responsible for the early phase of the blood pressure rise even with the presence of adiposity and the elevated leptin that made little contribution. We confirmed the rapid deposition of visceral fat by 2 independent methods, including dual-energy x-ray absorptiometry scanning, which showed a nearly 4-fold increase in fat mass with no change in lean muscle mass or bone density. Although the body weight gain is small on a HFD, the amount of fat rapidly expands in parallel with the elevated plasma leptin observed at 1 week of a HFD. In our present study showed that effectiveness of the leptin antagonist was associated with the emergence of a central pressor and sympathoexcitatory effect of leptin, an effect we previously found markedly elevated plasma leptin levels. Together these findings suggest that the activation of RSNA is not dependent on the current circulating levels of leptin but more on the presence of high circulating levels of leptin for a considerable period of time (several weeks). This finding is in accordance with our previous study that found that the hypertension and high RSNA persisted long after ceasing of the HFD and the normalization of circulating levels of leptin. In that study we postulated that the persistence of the hypertension and high RSNA levels in the presence of normal levels of insulin and leptin may be attributable to central actions of other adipokines or elevated plasma fatty acids. However, this now seems unlikely with the current findings where insulin seems mainly responsible for the early phase of the blood pressure rise even with the presence of adiposity and the elevated leptin that made little contribution. We confirmed the rapid deposition of visceral fat by 2 independent methods, including dual-energy x-ray absorptiometry scanning, which showed a nearly 4-fold increase in fat mass with no change in lean muscle mass or bone density. Although the body weight gain is small on a HFD, the amount of fat rapidly expands in parallel with the increased plasma leptin observed at 1 week of a HFD.

The question remains as to why there is a delay in the dependence of HFD-induced sympathoexcitation on central leptin signaling once leptin levels are increased (current study) and a delay in the recovery once leptin levels fall (previous study). Thus, there is no simple relationship between elevated peripheral levels of leptin emerging from visceral fat and the central

![Figure 4. Effects of intracerebroventricular administration of the vehicle, Ringer’s solution, on mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA, normalized units) after 1-week high-fat diet (HFD, gray circles) or normal diet (ND, open circles, left) or 3-week HFD (black circles) or ND (open circles, right). Data were averaged over 15-minute intervals. Right, Average changes (Δ) over 90 minutes after administration of Ringer’s (dotted line). Changes were calculated from initial Ringer’s (R) injection in rabbits on ND (open bar) and HFD (closed bars). Error bars are SEM or SE of the difference indicating variance between animals. C1 indicates control 1; and C2, control 2.](image-url)
signaling to produce sympathoexcitation. The appetite regulatory signaling has been quite well studied and involves activation of leptin receptors within the arcuate nucleus to inhibit appetite via activating proopiomelanocortin neurons and inhibiting neuropeptide Y neurons that project to other regions of the hypothalamus, such as the paraventricular nucleus and anterior and lateral hypothalamus. However, the pathways that mediate leptin signaling to increase SNA are less clear, although they likely involve similar hypothalamic pathways and neurons. Leptin may also act in multiple hypothalamic regions as demonstrated by direct microinjection of leptin in the dorsomedial medulla in rats to reduce blood pressure and in the ventrolateral hypothalamus to reduce both blood pressure and RSNA.

At this stage we can only speculate about the reason for the delayed contribution of central leptin to the sympathoexcitation and hypertension, although it does seem to coincide with the occurrence of leptin resistance, which we have observed in rabbits after 3 weeks of HFD when the number of Fos-activated neurons induced by central leptin administration is markedly reduced. The suppressor of cytokine signaling 3 is a negative-feedback regulator of leptin signaling involved in leptin resistance. However, the increased sensitivity to leptin and its contribution to increased RSNA cannot readily be explained by the lack of development of an inhibitory signal, such as suppressor of cytokine signaling 3. Perhaps more relevant is the time course of changes to hypothalamic signaling in mice during a HFD, which shows that proopiomelanocortin mRNA increases at 2 weeks but not at 1 week. Other neuropeptide signals, such as neuropeptide Y, agouti-related protein, orexin, and suppressor of cytokine signaling 3, are not altered.

The present study importantly investigated the contribution of central insulin to the hypertension and activation of RSNA during a short-term HFD given to conscious rabbits. ICV injection of a specific insulin antagonist reduced blood pressure and HR equally at both 1 and 3 weeks but there was little effect on RSNA. The effects were less than the effect of leptin at 3 weeks. These findings suggest that the initial increase in blood pressure early on in a HFD when the hypertension is modest is largely driven by central actions of insulin. These effects persist but by 3 weeks, clearly the cumulative influence of circulating leptin and the increased sensitivity to its central actions are greater than that of insulin.

Insulin is taken up into the brain from the blood stream by a receptor-mediated transport process. It functions as a peripheral regulator of nutrient storage and release as well as a key afferent signal to the central nervous system for energy balance. Insulin receptors are expressed in several regions of the central nervous system, with a high density in the hypothalamus. Neurons in the arcuate nucleus that express proopiomelanocortin and others that express neuropeptide Y, both express insulin receptors. However, the surprising finding was that although the central effects of the insulin antagonist were clear early on in those animals on a HFD, there was no effect of the insulin antagonist on RSNA. Similar to our findings, Vaz et al have reported that in obese subjects, although fasting serum insulin concentrations are higher, serum insulin and renal noradrenaline spillover values are not quantitatively related, which shows that hyperinsulinemia per se does not lead to elevated RSNA. Furthermore, ICV infusion of insulin to rats increases lumbar SNA but not RSNA. Together with our finding, it would seem that the early increase in blood pressure to diet-induced elevation of insulin signaling may activate sympathetic vasomotor activity to beds other than the kidney. An additional suggestion is that the hypotension we observed to the central insulin antagonist was a result of reduced cardiac output, possibly related to the reduction in HR or in venous return (both can be decreased by inhibiting sympathetic activity). There is an established relationship between the ultradian rhythms of insulin secretion and the rhythms of autonomic function, presumably to cope with the metabolic load changes associated with the sleep/active cycle and to also coincide with the higher post prandial glucose. Thus, there is a conjunction between the metabolic and cardiovascular needs of high activity periods linked through hypothalamic circuitry, perhaps involving cardiac sympathoexcitation (based on the known actions of central insulin on baroreflexes in rats). We have reported a strong relationship between the changes in glucose levels and HR in a previous study of rabbits on a HFD. Thus, it follows that the HFD, being high in calories and leading to higher levels of plasma glucose and insulin, may inappropriately signal higher levels of HR and hence cardiac output and blood pressure at the level of the hypothalamus.

Interestingly, we did not see a reduction in HR with leptin at 3 weeks. However, this was in the presence of a rapid large fall in blood pressure, which would normally induce a reflex tachycardia. We have previously shown that the HR baroreflex curve shifts to the right and the upper plateau increases after 3 weeks of HFD. Thus, the leptin antagonist may reverse these changes and by altering the baroreflex curve allows for larger changes in blood pressure without influencing HR.

A strength of our current study was that we documented the effectiveness of the leptin and insulin antagonists. In separate experiments we used dose–response curves to determine initially the optimum pressor effect of leptin and insulin (at 3 weeks and 1 week of a HFD, respectively) and then pretreated rabbits ICV with the same dose of the antagonist before the agonist. We found that the pressor responses to leptin and insulin were abolished by the antagonist pretreatment. We also included control ICV injections of Ringer’s solution, which had no effects on MAP, HR, and RSNA in both ND and HFD rabbits.

Perspectives
We have now shown that the elevation of blood pressure and RSNA induced by a HFD for several weeks is predominantly mediated by central actions of leptin. Furthermore, central actions of insulin contribute a smaller proportion of the hypertension initially, presumably through sympathoexcitation but independently of RSNA. The rapid cardiovascular response to insulin elevation (or decrease after removal of a HFD) may reflect the role of insulin in the diurnal pattern of responding to circulating glucose and the metabolic load changes associated with the sleep/active cycle and to also coincide with the higher post prandial glucose. By contrast, there is a considerable delay in the renal sympathoexcitatory effects of leptin, which may allow for short-term regulation of sodium excretion in relatively limited periods of feast and famine. Future studies are now aimed at understanding
why elevation of leptin for several weeks eventually leads to increased RSNA and blood pressure.

Sources of Funding
This work was supported by a grant from the National Health and Medical Research Council of Australia (Project 526618). The study was supported in part by the Victorian Government’s Operational Infrastructure Support Program. Geoffrey A. Head was funded by a grant from the National Health and Medical Research Council of Australia Fellowship (APP1002186).

Disclosures
None.

References
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Hypertension. 2013;61:628-634; originally published online January 21, 2013; doi: 10.1161/HYPERTENSIONAHA.111.00705
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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OBESITY RELATED HYPERTENSION AND THE ROLE OF INSULIN AND LEPTIN IN HIGH FAT FED RABBITS

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Running Title: Insulin and leptin in obesity related hypertension

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Methods

Tissue collection and assessment of body composition

Rabbits were killed by an anesthetic overdose (160 mg/kg IV, sodium pentobarbitone, Virbac, Milperra, NSW, Australia) at the end of week 1 or week 3. Body fat (white and brown adipose tissue pads, dissected from the perirenal, omental and epidydimal areas and between the scapulae) were collected and weighed. Lean muscle mass, bone mineral content and total % of fat were determined by scanning with a bone densitometer (Hologic Discovery A-QDR, Inc. MA USA).

Results

Table S1 Body composition determined using dual-energy X-ray absorptiometry scanning (Dexa) and fat mass harvested manually at post mortem.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>HFD Week 1</th>
<th>HFD Week 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral content (g)</td>
<td>58.4 ± 3.9</td>
<td>66.2 ± 2.8</td>
<td>61.0 ± 2.6</td>
<td>0.162</td>
<td>0.569</td>
<td>0.355</td>
</tr>
<tr>
<td>Lean muscle (g)</td>
<td>3107 ± 152</td>
<td>3054 ± 65</td>
<td>3066 ± 66</td>
<td>0.763</td>
<td>0.796</td>
<td>0.948</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>48.8 ± 17.6</td>
<td>161.0 ± 31.2</td>
<td>183.6 ± 19.6</td>
<td>0.002</td>
<td>0.000</td>
<td>0.496</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>2.4 ± 0.3</td>
<td>4.9 ± 0.8</td>
<td>6.7 ± 0.5</td>
<td>0.002</td>
<td>0.000</td>
<td>0.022</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>HFD Week 1</th>
<th>HFD Week 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass (manually collected)</td>
<td>41.2 ± 8.5</td>
<td>126.4 ± 12.5</td>
<td>139.9 ± 18.7</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Fat mass per BWT

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>HFD Week 1</th>
<th>HFD Week 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass per BWT</td>
<td>1.4 ± 0.2</td>
<td>3.9 ± 0.4</td>
<td>4.5 ± 0.6</td>
<td>0.000</td>
<td>0.000</td>
<td>0.286</td>
</tr>
</tbody>
</table>

Values are mean and SEM in rabbits on a normal diet and rabbits on high fat diet (HFD) for 1 week and for 3 weeks. P1 is probability for comparison between normal and HFD 1 week groups, P2 is probability for comparison between normal and HFD 3 week groups, P3 is probability for comparison of HFD 1 week and 3 week groups and shown in italics if P < 0.05.