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Rapid Onset of Renal Sympathetic Nerve Activation in Rabbits Fed a High-Fat Diet

James A. Armitage, Sandra L. Burke, Larissa J. Prior, Benjamin Barzel, Nina Eikelis, Kyungjoon Lim, Geoffrey A. Head

Abstract—Hypertension and elevated sympathetic drive result from consumption of a high-calorie diet and deposition of abdominal fat, but the etiology and temporal characteristics are unknown. Rabbits instrumented for telemetric recording of arterial pressure and renal sympathetic nerve activity (RSNA) were fed a high-fat diet for 3 weeks then control diet for 1 week or control diet for 4 weeks. Baroreflexes and responses to air-jet stress and hypoxia were determined weekly. After 1 week of high-fat diet, caloric intake increased by 62%, accompanied by elevated body weight, blood glucose, plasma insulin, and leptin (8%, 14%, 134%, and 252%, respectively). Mean arterial pressure, heart rate, and RSNA also increased after 1 week (6%, 11%, and 57%, respectively). Whereas mean arterial pressure and body weight continued to rise over 3 weeks of high-fat diet, heart rate and RSNA did not change further. The RSNA baroreflex was attenuated from the first week of the diet. Excitatory responses to air-jet stress diminished over 3 weeks of high-fat diet, but responses to hypoxia were invariant. Resumption of a normal diet returned glucose, insulin, leptin, and heart rate to control levels, but body weight, mean arterial pressure, and RSNA remained elevated. In conclusion, elevated sympathetic drive and impaired baroreflex function, which occur within 1 week of consumption of a high-fat, high-calorie diet, appear integral to the rapid development of obesity-related hypertension. Increased plasma leptin and insulin may contribute to the initiation of hypertension but are not required for maintenance of mean arterial pressure, which likely lies in alterations in the response of neurons in the hypothalamus. (*Hypertension*. 2012;60:163-171.) • [Online Data Supplement](#)

Key Words: sympathetic nervous system ■ obesity ■ rabbits ■ blood pressure ■ heart rate

Obesity represents a significant risk for cardiovascular disease because of the relationship between excess body fat and hypertension. It is estimated that obesity contributes to hypertension in >60% of men and women entering the Framingham study.¹ The mechanisms underlying this relationship are multifactorial, and for some time there was controversy as to whether the sympathetic nervous system was activated or inhibited in obesity-related hypertension. Bray² proposed that obesity was a result of low thermogenic activity secondary to low sympathetic activity, and certainly data from heart rate (HR) variability studies supported this hypothesis. Young and Landsberg³ hypothesized that sympathetic outflow is increased in obesity to facilitate energy wastage by thermogenesis and to maintain body weight homeostasis, with elevated renal sympathetic activation and hypertension the sequelae. It is now clear that norepinephrine spillover to renal and skeletal muscle beds is increased in obese humans,⁴ and microneurographic data indicate that skeletal muscle sympathetic nerve activity is greater in

overweight humans,⁵ consistent with the observation that sympathetic vasomotor activity in skeletal muscle is elevated in established obesity.^{6,7} The sympathoexcitation is associated with long-term obesity and the accumulation of body fat.⁸

We have shown that obesity-related hypertension is characterized by elevated renal sympathetic nerve activity (RSNA) in fat-fed rabbits and that the strongest predictors of sympatho-excitation and hypertension are visceral fat deposits and leptin.⁹ Using the same high-fat diet with rabbits, Antic et al¹⁰ showed that the elevation in blood pressure occurred as rapidly as 1 week when the increase in body weight is <10%. If the mechanism of the hypertension was related to raised RSNA in response to plasma leptin, then both should occur within 1 week and remain high if the animals are returned to a normal diet. The advent of telemetry for RSNA has enabled us to determine the rapidity of sympathetic activation and its association with hypertension in a rabbit model of obesity, which is the major aim of the current study.

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The second aim of our study was to determine whether sympathetic activation during the early development of obesity involves dysregulation of chemoreflexes and stress inputs. Obesity-related hypertension is frequently associated with obstructive sleep apnea in which intermittent episodes of hypoxia produce an ongoing elevation in sympathetic nerve activity, even when the hypoxia is not present.^{11,12} Moreover, obese subjects have exaggerated neural responses to stressful stimuli,¹³ as well as impaired baroreflexes.¹⁴ Thus, we examined the changes in the responses to arterial hypoxia, acute stress, and in baroreflex function during the early phase of the high-fat diet (HFD) to identify whether altered chemoreflexes, stress reactivity, and baroreflexes occur in the development phase of obesity-related hypertension or if they are secondary pathologies.

Methods

Experiments were conducted in 33 male New Zealand white rabbits (body weight, 2.6–3.3 kg) housed under controlled conditions of light (6:00 PM to 6:00 AM), temperature, and humidity. Experiments were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Rabbits underwent preliminary surgical operations under isoflurane anesthesia to implant an aortic blood pressure cannula connected to a radiotelemetry transmitter (model TA11PA-D70, Data Sciences International, St Paul, MN) and renal nerve electrode connected to a radiotelemetry (model TR76S or TR46S, Telemetry Research, Auckland, New Zealand). Carprofen (3 mg · kg⁻¹) was given before and 24 hours after surgery for analgesia.

After 1-week recovery, baseline mean arterial pressure (MAP), HR, and RSNA were measured in the laboratory. MAP measured by telemetry was calibrated to MAP measured via an indwelling ear artery catheter. Responses to air-jet (a stream of air directed for 10 minutes at the rabbit's nose at 60 L · min⁻¹) and the last 10 minutes of hypoxia (10% O₂ and 3% CO₂ in N₂ for 20 minutes at 10 L · min⁻¹) were assessed, with 30 minutes of recovery in between. RSNA and HR baroreflexes were derived in duplicate from slow ramp rises and falls in MAP induced by IV infusions of 0.5 mg · mL⁻¹ of phenylephrine (25 µg · kg⁻¹) and 1 mg · mL⁻¹ of sodium nitroprusside (30 µg · kg⁻¹), respectively.¹⁵ Arterial blood samples (4 mL) were taken for glucose measurement by glucometer (Optium Xceed, Abbott, Doncaster, Victoria, Australia) and plasma stored at -80°C for analysis of insulin (high-sensitivity ELISA with rabbit insulin standard, CrystalChem Downers Grove, IL) and leptin by radioimmunoassay using a multispecies kit (LINCO Research, St Charles, MO).⁹ Rabbits were then randomized into 2 groups and meal fed 110 g of a normal fat diet (control; n=10) or 190 g of HFD (n=13) for 3 weeks. The control diet was standard rabbit chow (4.2% fat, 2.63 kcal/g, SF06-011, Specialty Feeds, Glen Forrest, Western Australia, Australia) and the HFD was modified standard rabbit chow (13.3% fat, 3.34 kcal/g, SF02-005, Specialty Feeds).⁹ The HFD was replaced by the control diet for the last week of the 4-week treatment protocol to assess the acute effects of withdrawal of the HFD. Rabbits were weighed weekly, and food intake was measured in a subset of rabbits (n=4–5 per group) to calculate calorie consumption. Experiments to measure MAP, HR, and RSNA; responses to air-jet and hypoxia; and baroreflexes were conducted at weekly intervals. At week 3, the maximum fall in MAP to ganglion blockade (pentolinium tartrate, 6 mg · kg⁻¹, Sigma, St Louis, MO) was assessed in a subset of animals (n=4–5 per group). At the end of the final experiment, white adipose tissue pads were dissected from the omentum and mesenteric viscera, the perirenal area, epididymis, and bladder and weighed. Heart, kidneys, adrenal glands, liver, and interscapular brown adipose tissue were also

Table. Baseline Values in Rabbits Fed a Control or HFD

Variable	Control	HFD
Body weight, kg	3.14±0.06	3.14±0.05
Blood glucose, mmol · L ⁻¹	6.1±0.2	5.9±0.1
Plasma insulin, ng · mL ⁻¹	0.51±0.03	0.44±0.03
MAP, mm Hg	72±1	70±1
Heart rate, beats · min ⁻¹	172±3	176±4
RSNA, normalized units	7.3±0.5	5.5±0.3
RSNA, µV	34.4±3.3	23.3±1.4*
Nasopharyngeal response, µV	524.6±54.9	461.0±37.8

Values are mean±SEM. HFD indicates high-fat diet; MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity (normalized units).

**P*<0.01 for comparison of control with HFD.

collected and weighed. In a separate group of rabbits (n=10), lean muscle mass, bone mineral content, and total percentage of fat were determined after 1 or 3 weeks of HFD or control diet by scanning with a dual energy X-ray densitometer (Hologic Inc).

MAP and HR, derived from the pressure pulse, and RSNA were digitized at 500 Hz using an analog-to-digital data acquisition card (National Instruments 6024E) and averaged over 2 seconds. RSNA was scaled to 100 normalized units by the maximum nasopharyngeal response evoked by smoke, given at weekly intervals.¹⁶ RSNA responses were measured during 3 weeks of treatment in control (n=8) and HFD (n=6) rabbits, but only 3 to 4 rabbits per group had viable RSNA at week 4 because of the decay of the signal after ≈5 weeks of recording. Resting levels of all of the variables were measured over 30 minutes. MAP-RSNA and MAP-HR baroreflex curves were fitted by a 5-parameter logistic function.¹⁷ Values are expressed as mean±SEM or mean difference±SE of the difference. Data were analyzed by split-plot repeated-measures ANOVA that allowed for within-animal and between-animal (group) contrasts. One-way ANOVA was used for data collected at a single time point. Type 1 error was controlled using Bonferroni and Greenhouse-Geisser corrections.¹⁸ A probability of *P*<0.05 was considered significant.

Results

Effect of the HFD Over 3 Weeks and Return to Normal Diet

Body weight before beginning the diets did not differ between groups (Table). Rabbits fed the HFD (n=13) gained 260±35 g during the first week and continued to gain weight over the next 2 weeks (504±43 g at week 3) compared with control rabbits, which had gained 80±26 g by week 3 (n=10; *P*_{group}<0.001; Figure 1). During the first week of HFD, caloric intake increased by 62% from 299±4 kcal · d⁻¹ but declined from then until week 3 when intake was 42% greater than baseline (*P*<0.001; n=5; Figure 1). Control rabbit intake did not increase over the 3 weeks of measurement (n=4; Figure 1). Blood glucose and plasma insulin concentrations were initially similar in the 2 groups (Table) but increased significantly during the first week of the HFD (+14% and +134%, respectively; *P*<0.001) and remained elevated for the next 2 weeks (Figure 1). Blood glucose and plasma insulin concentrations in control rabbits were unchanged over 3 weeks (*P*_{time}>0.05<0.001; Figure 1). Insulin sensitivity, estimated by the glucose/insulin ratio, showed a rapid decline with HFD consumption and was 47% lower than baseline during weeks 1 to 3 of the HFD (*P*_{group×diet}=0.042). Plasma leptin concentrations increased markedly at week 1

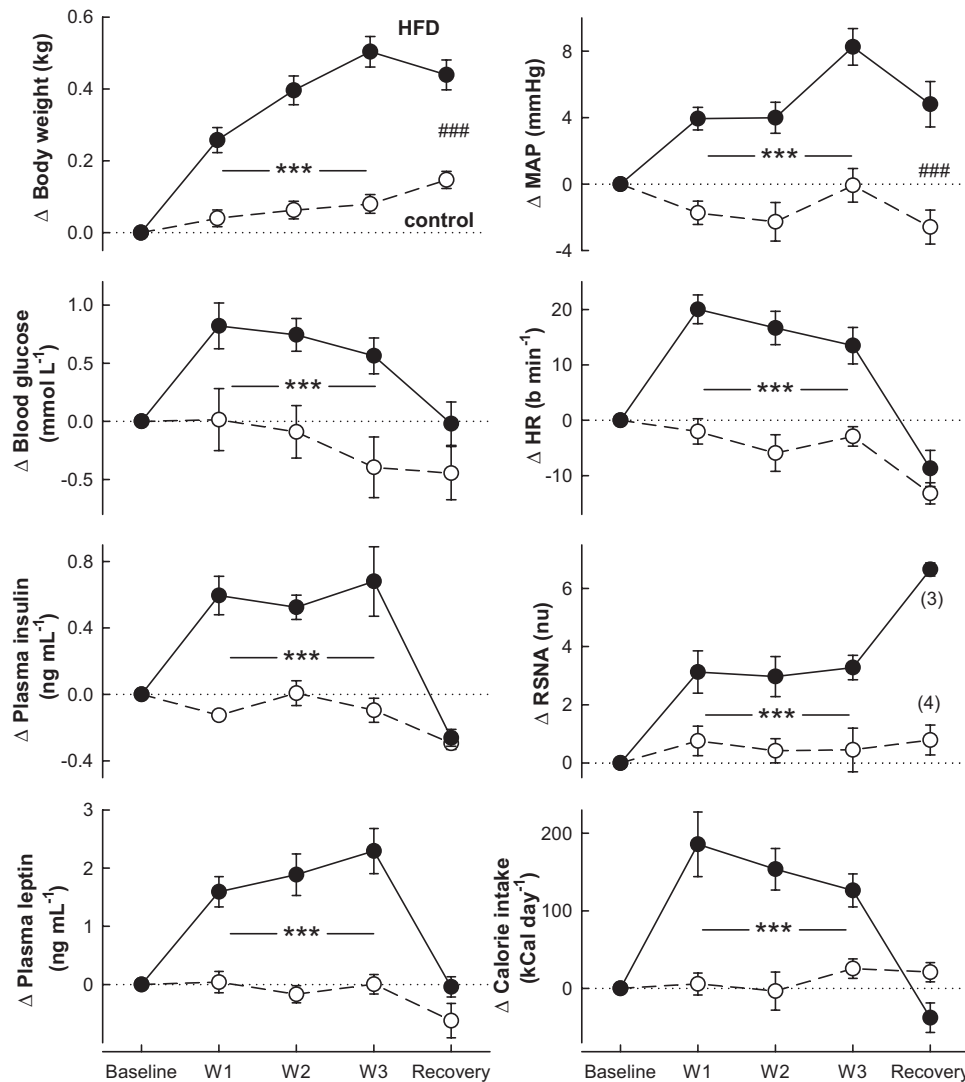


Figure 1. Changes from baseline in body weight, blood glucose, plasma insulin concentration, plasma leptin concentration, calorie intake, mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA, normalized units [nu]) of rabbits fed a normal diet (○, n=10) or high-fat diet (HFD; ●, n=13). Parameters were measured weekly for 3 weeks after the commencement of the diet (week 1 through week 3) and for 1 week after cessation of the diet (recovery). Caloric intake was measured in 4 control and 5 HFD rabbits; plasma insulin was measured in 5 rabbits in each group; plasma leptin was measured in 4 control and 8 HFD rabbits; RSNA was measured in 8 control and 6 HFD rabbits to week 3, and in 3 to 4 from each group at recovery (indicated on graph). Values are mean difference \pm SE of the difference indicating variance between animals. *** $P < 0.001$ for control vs HFD at weeks 1 to 3; ### $P < 0.001$ for control vs HFD at recovery.

(+252%; $P < 0.001$) and remained elevated throughout the HFD, whereas plasma leptin concentration in control rabbits did not change over the same period (Figure 1).

Baseline MAP and HR were also similar between groups (Table). MAP increased by 6% during the first week of HFD ($P < 0.001$), but by week 3 that increase had doubled to 12% (Figure 1). HR, by contrast, increased by 11% at week 1 ($P < 0.001$) and remained at a similar level during the following 2 weeks (Figure 1). There was a marked increase in RSNA at week 1 of the HFD ($+3.1 \pm 0.7$ normalized units; $P < 0.001$), which was maintained throughout the treatment period (Figure 1). By contrast, MAP, HR, and RSNA in rabbits fed a control diet did not change significantly over the 3-week treatment period ($P_{\text{time}} > 0.05 < 0.001$; Figure 1). The nasopharyngeal response that was used to

normalize RSNA was not altered by time on the HFD ($P_{\text{group} \times \text{diet}} = 0.58$).

One week after resuming a normal diet, calorie intake had fallen to a level similar to that of control rabbits, accompanied by falls in blood glucose, plasma insulin, and plasma leptin concentrations, as well as HR (Figure 1). Insulin sensitivity showed a large rebound in the first week after return to a normal diet (+265%). However, body weight and MAP in rabbits previously fed the HFD remained elevated compared with control rabbits ($P_{\text{groups}} < 0.001$), although MAP was 4% lower than the peak response ($P = 0.002$ for week 3 versus recovery; Figure 1). Interestingly, the 3 rabbits previously on the HFD, which had working electrodes 1 week after return to a normal diet, demonstrated maintenance of the high RSNA. Control animals showed no change

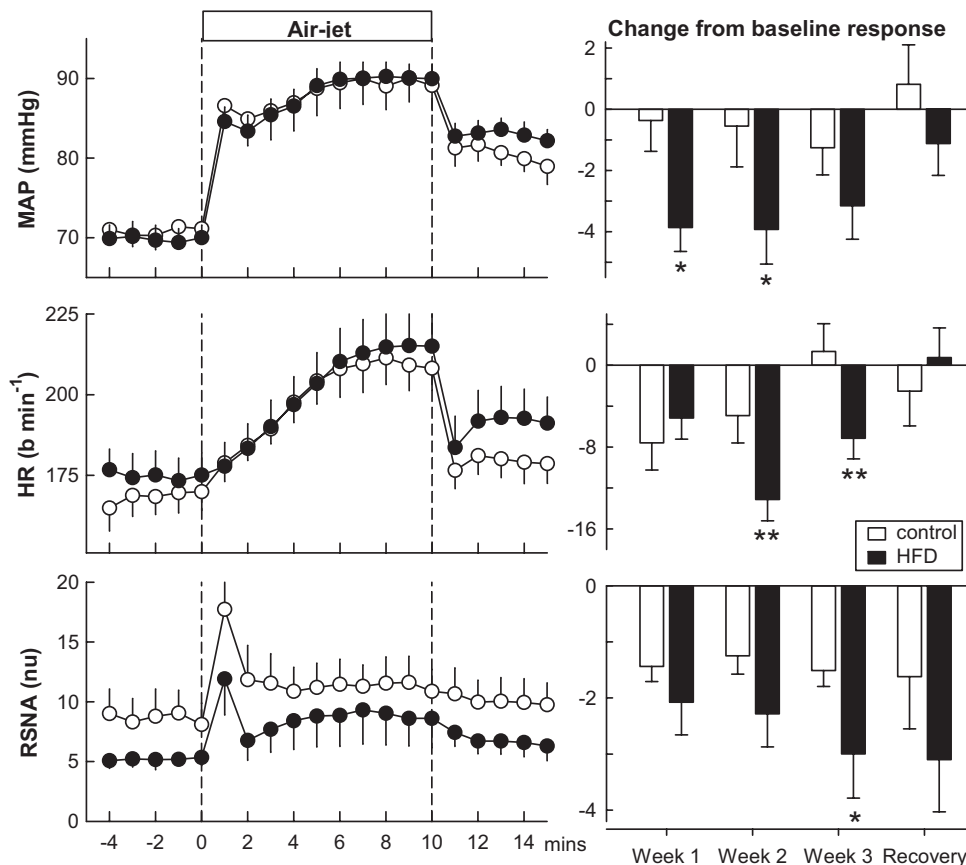


Figure 2. Left, Effects of exposure to air-jet stress before control diet (○) or high-fat diet (HFD; ●). Data are 1-minute averages of mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA; normalized units) measured for 5 minutes before, 10 minutes during, and 5 minutes after air-jet stress. Error bars are SEM indicating between-animal variance. Right, Average changes from baseline response of the same parameters measured during 10 minutes of air-jet stress for 3 weeks after commencement of HFD and 1 week after cessation of the HFD (recovery). □, control; ■, HFD. Error bars are SE of the difference indicating between-animal variance. * $P < 0.05$ and ** $P < 0.01$ for comparison of control rabbits with those fed the HFD. RSNA data were recorded in $n = 5$ to 6 rabbits in weeks 1 to 3 and $n = 3$ to 4 rabbits in recovery week.

in RSNA over the course of the experimental schedule (Figure 1).

Effect of HFD and Return to Normal Diet on Responses to Air-Jet Stress

Rabbits were exposed to an air-jet before and at weekly intervals after initiation of the HFD. Initial responses to the air-jet stimulus were similar in the 2 groups and consisted of a rapid increase in MAP ($+18 \pm 1$ mm Hg), tachycardia ($+29 \pm 2$ beats \cdot min⁻¹) and sympathoexcitation ($+3.5 \pm 0.4$ normalized units). In control rabbits ($n = 8$), the MAP and HR responses to air-jet stress over 3 weeks were similar to those observed during the baseline experiment (Figure 2). By contrast, after the onset of the HFD ($n = 11$), the MAP responses to air jet were reduced by 21% (weeks 1–2; $P < 0.05$) and the tachycardia reduced by 26% to 48% (weeks 2–3) compared with baseline ($P < 0.01$; Figure 2). Sympathoexcitation in response to air-jet stress was reduced in both control and HFD rabbits compared with the baseline response (Figure 2). However the reduction became progressively greater in the HFD group over the course of 3 weeks (-70% at week 3; $P_{\text{time}} < 0.05$), whereas we did not observe any further change in the response in the control group (Figure 2).

After 1 week of control diet in rabbits previously fed the HFD, both the MAP and HR responses to air-jet stress were restored to baseline levels and were similar to responses in control rabbits (Figure 2). After the return to control diet, there was no significant difference between RSNA responses in the 2 groups ($n = 3$ –4; Figure 2).

Effect of HFD and Return to Normal Diet on Responses to Hypoxia

The cardiovascular and RSNA responses to hypoxia were measured before and at weekly intervals after beginning the HFD. At the baseline experiment, rabbits in control ($n = 10$) and HFD ($n = 13$) groups showed characteristic pressor ($+4.5 \pm 0.4$ mm Hg), tachycardic ($+18 \pm 1$ beats \cdot min⁻¹), and sympathoexcitatory ($+2.5 \pm 0.3$ normalized units) responses to hypoxia. In control rabbits and in rabbits fed the HFD, we observed no change in the responses to hypoxia over the following 3 weeks (Figure 3). Consumption of the HFD and then return to a normal diet did not significantly alter these responses ($P_{\text{group}} > 0.05$; Figure 3).

Effect of HFD and Return to Normal Diet on Baroreflexes

RSNA and HR baseline baroreflex curves were sigmoidal, with resting values lying close to the lower plateau. In control

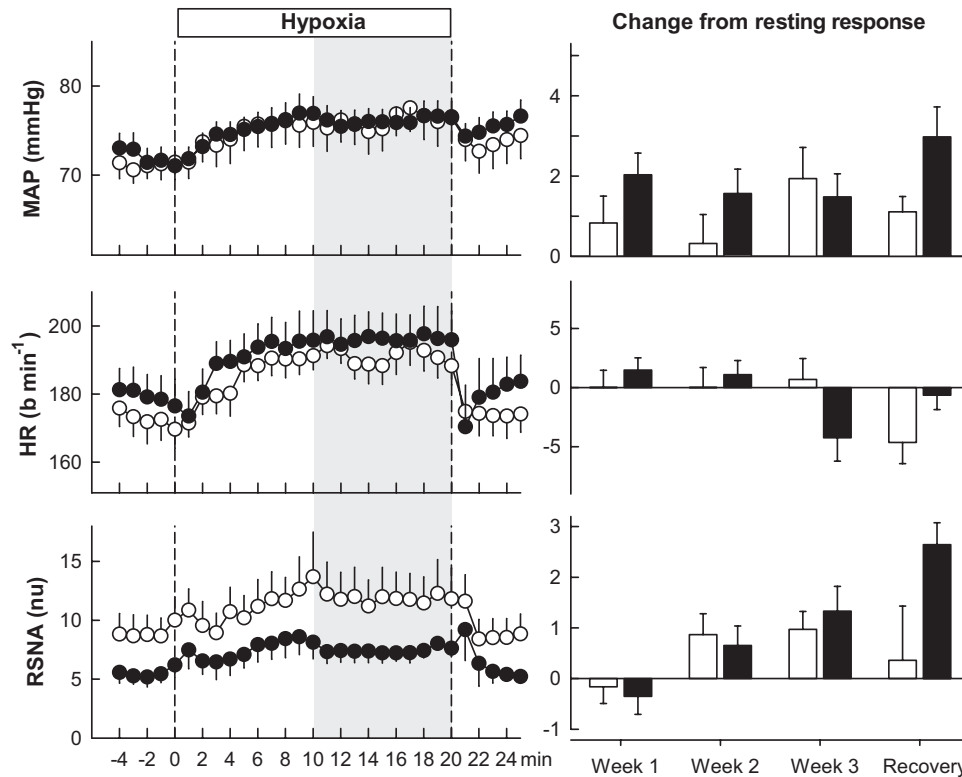


Figure 3. Left, Effects of exposure to hypoxia before control diet (○) or high-fat diet (HFD; ●). Data are 1-minute averages of mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA; normalized units) measured for 5 minutes before, 20 minutes during, and 5 minutes after hypoxia. Error bars are SEM indicating between animal variance. Right, Average changes from baseline response of the same parameters measured during the last 10 minutes of hypoxia (shaded gray in left panel) for 3 weeks after commencement of HFD and 1 week after cessation of the HFD (recovery). Error bars are SE of the difference indicating between-animal variance. RSNA data recorded in n=6 to 8 rabbits in weeks 1 to 3 and n=3 to 4 rabbits in recovery week.

rabbits, we observed no significant differences between RSNA baroreflex curves at baseline and those constructed over the following 3 weeks (Figure 4 and Table S1, available in the online-only Data Supplement). HR baroreflexes measured in control rabbits, which were repeated 4 times at weekly intervals, were also very similar to those produced at the baseline experiment (Figure 5 and Table S2).

In rabbits fed the HFD, there was a marked progressive increase in the lower plateau of the RSNA baroreflex, from week 1 until week 3, when the minimum RSNA was 92% higher than at baseline ($P<0.001$; Figure 4 and Table S1). This resulted in an attenuation of the baroreflex range (29% at week 3; $P<0.001$), because there was little change in the upper plateau over this time period (Figure 4 and Table S1). RSNA baroreflex gain, which depends on range, was also markedly reduced at weeks 2 and 3 (−48% at week 3; $P=0.003$; Figure 4 and Table S1). By contrast, the main effect of the HFD on the HR baroreflex was to shift the curve in the direction of the MAP increase (8% increase in blood pressure at half the reflex range at week 3; $P<0.001$; Figure 5 and Table S2). There was initially an attenuation of the curve at week 1, with an elevation of the lower plateau and reductions in reflex range and gain compared with baseline (Figure 5 and Table S2). However, by week 3, the HR baroreflex upper plateau and reflex range had recovered and were greater than baseline (6% to 8%; $P<0.05$), and there was restoration of baroreflex gain toward the baseline value

($P=0.2$; Figure 5 and Table S2). HR baroreflex curves constructed 1 week after rabbits on the HFD were returned to a normal diet were still enhanced, most notably baroreflex gain, which was 33% greater than at week 3 ($P<0.001$) but not different from baseline (Table S2).

Effect of HFD on MAP Response to Ganglion Blockade

Ganglion blockade in rabbits fed the HFD for 3 weeks produced a fall in MAP (-23 ± 2 mm Hg; n=5), which was almost double that observed in control rabbits (-13 ± 2 mm Hg; n=4; $P_{\text{group}}=0.014$).

Effect of HFD and Return to Normal Diet on Fat Pad and Organ Mass

Over the 3 weeks of fat feeding, rabbits developed an increase in adipose tissue mass distributed across perirenal, mesenteric, testicular, bladder white adipose tissue depots ($P_{\text{group}}<0.001$), and brown adipose tissue depots ($P_{\text{group}}<0.01$), which was maintained after 1 week of normal diet. Body composition data are given in Table S3.

Discussion

These data show that elevated RSNA occurs very early in the development of obesity-related hypertension, and ganglionic blockade data suggest that much of the observed hypertension is attributable to elevated sympathetic activity. Within 1 week

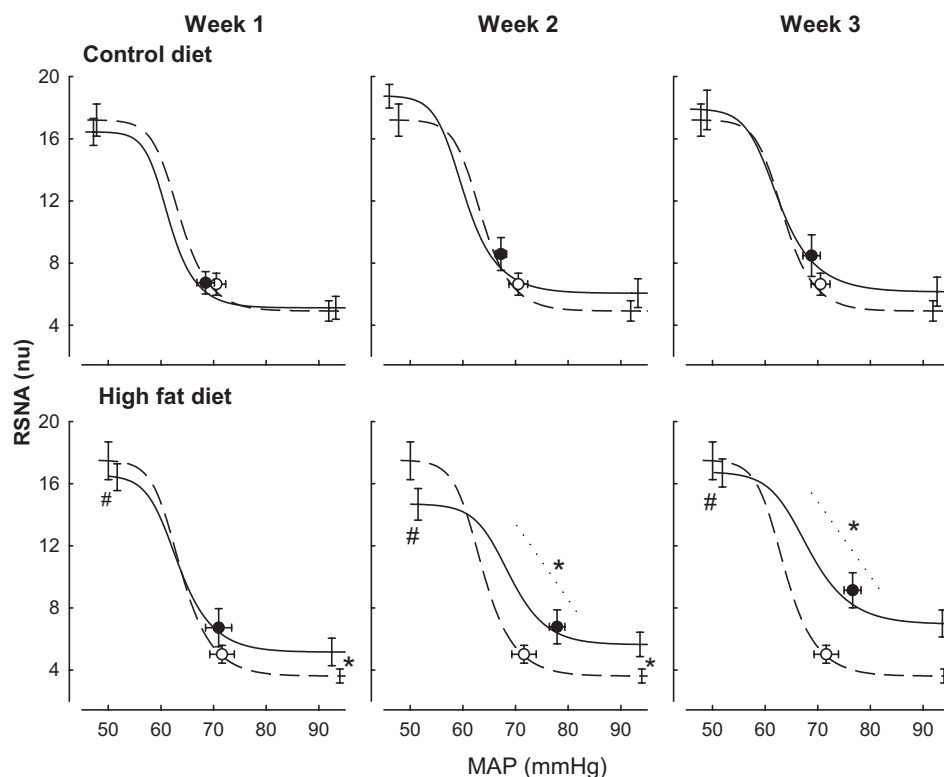


Figure 4. Renal sympathetic nerve activity (RSNA; normalized units) baroreflex curves before (dashed lines) and at weeks 1, 2, and 3 (solid lines) after commencement of control (upper, $n=7$) or high-fat (lower, $n=6$) diet. Symbols on curves represent resting values. Error bars are SEM, indicating between-animal variance. * $P<0.05$ for baseline vs week for gain (indicated by dotted line) and lower plateau; # $P<0.05$ for baseline vs week for range.

of commencing the HFD, blood pressure, HR, and RSNA were elevated, and this was associated with attenuation of the RSNA baroreflex and of the MAP response to stress. Continuation of the HFD for another 2 weeks further increased MAP and further attenuated the RSNA baroreflex and stress responses, but RSNA and HR did not change. Although we have established previously that RSNA is elevated with established obesity or weight gain, in this study we have serially measured RSNA in animals as they develop obesity and hypertension, supporting the hypothesis that sympathetic drive is increased rapidly on consumption of a high calorie diet and is central to obesity-related hypertension.

Rapid Changes in Cardiovascular Parameters With Short-Term Food Increase

Rabbits consuming the HFD ingested ≈ 200 kcal per day more than controls, which represents a 40% to 60% increase above basal energy intake. The elevation in HR and blood pressure in response to long-term caloric loads is characterized in human,¹⁹ canine,²⁰ rabbit,²¹ and rodent models.²² The novelty of our study relates to recording of RSNA during the very earliest stages of obesity-related hypertension. In humans, 8 weeks of fat gain (equating to ≈ 3.9 kg of body weight) results in elevated cardiac sympathetic activation, which is reversed on weight loss.¹⁹ In a canine model of obesity, 21 weeks of fat feeding was associated with hypertension, and HR variability studies indicated an early loss of parasympathetic tone and a gradual increase in cardiac sympathetic tone.²⁰

Our observation that HR increases within 7 days of consuming the HFD is consistent with the previous findings of Antic et al,²³ who showed that 7 days of high-fat, high-calorie intake resulted in an immediate increase in HR and a small but significant increase in MAP. Interestingly, longer term fat feeding does not appear to exacerbate the magnitude of the hypertension.¹⁰ Our present data now indicate that RSNA increases rapidly in the first week of fat feeding, well before the onset of frank obesity. The ganglionic blockade experiments offer clear evidence that the development of hypertension is attributed almost entirely to elevated whole body sympathetic tone, and although there is variation in sympathetic activity across the body it is likely that the 20% increase in RSNA would contribute significantly to this MAP elevation.

The mechanism driving this increase in RSNA is yet to be determined but may involve insulin, fatty acid metabolism, and leptin pathways. We have previously demonstrated a strong correlation between plasma leptin concentration and RSNA, hypertension, and tachycardia in rabbits within 3 weeks of fat feeding.⁹ In the present study, plasma insulin and leptin concentrations rose rapidly in the first week of high-fat feeding and were maintained at a similar magnitude in weeks 2 and 3. This pattern of increase mirrors that of HR, MAP, and RSNA, suggesting a possible role for insulin, as well as leptin, in the development of obesity-related hypertension. In support of this hypothesis, acute insulin infusion studies in humans indicate that elevated plasma insulin concentration

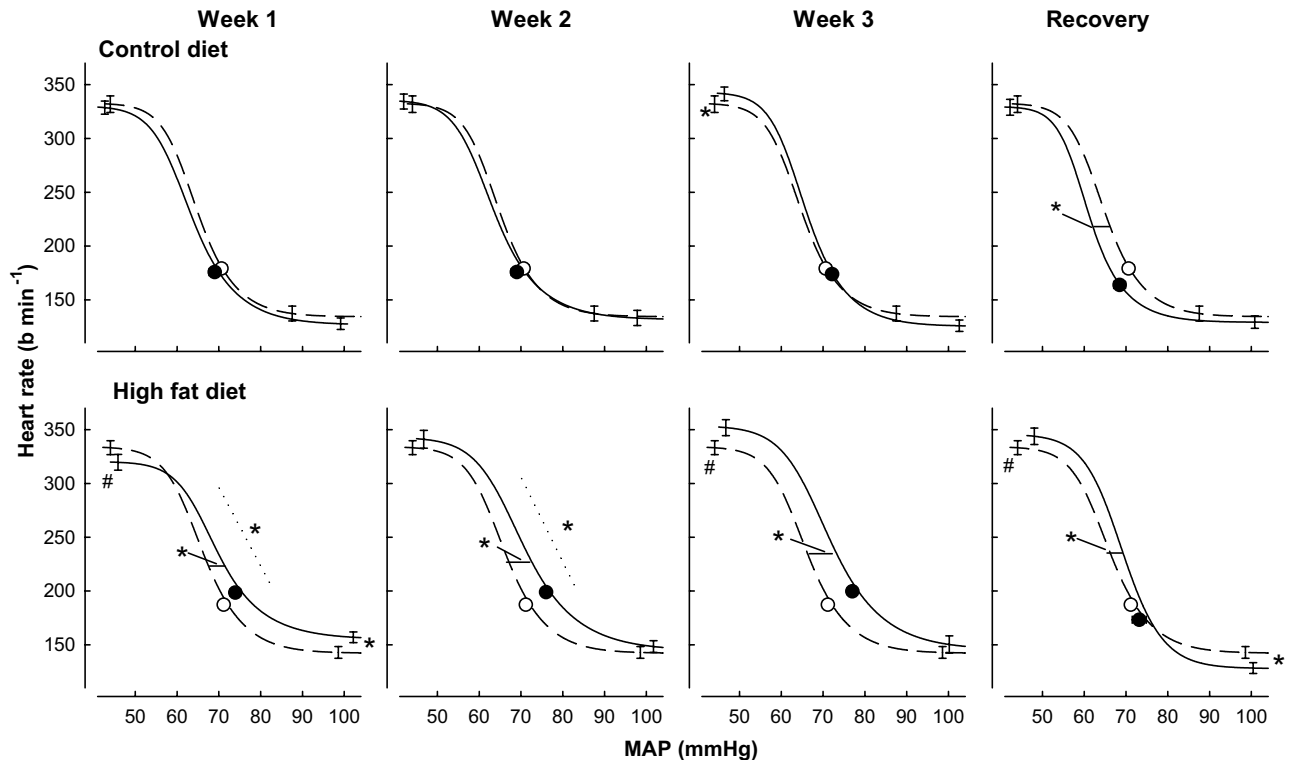


Figure 5. Heart rate baroreflex curves before (dashed lines) and at weeks 1, 2, and 3 and recovery (solid lines) after commencement of control (**upper**) or high-fat (**lower**) diet. Symbols on curves represent resting values. Error bars are SEM, indicating between-animal variance. * $P < 0.05$ for baseline vs week for gain (indicated by dotted line), lower plateau, and BP50 (blood pressure at half the reflex range); # $P < 0.05$ for baseline vs week for range; $\phi P < 0.05$ for baseline vs week for upper plateau.

is associated with elevated sympathetic activity in skeletal muscle.²⁴

The relationship among insulin, leptin, and MAP appears more complex than we have reported previously,⁹ because the rising trend of MAP follows that of insulin and leptin during the onset of obesity but does not appear to hold after the withdrawal of HFD, where plasma leptin and insulin concentrations fall rapidly but MAP and RSNA remain high. This novel observation suggests that the relationship among insulin/leptin concentrations and MAP and RSNA may have altered. An alternative explanation is that insulin may have actions early in the development of obesity-related hypertension, but then other factors maintain the hypertensive state, and insulin is not involved in the maintenance of obesity-related hypertension. A number of studies report a stable relationship between appetite-controlling peptide and cardiovascular arousal, and indeed our previous work⁹ supports this view. A possible explanation is that maintenance of elevated MAP and RSNA occurs because second-order neural pathways in the hypothalamus that have been conditioned over several weeks to be responsive to leptin or insulin remain so for a considerable period when the high levels of insulin and leptin have returned to normal. Another possibility is that other adipokines are still signaling centrally from the expanded fat deposits still present, which maintain the higher blood pressure and RSNA. Alternatively, the action of the HFD may be transduced by elevated plasma fatty acids. In healthy human subjects, an acute 4-hour infusion of fatty acids results in hypertension (+14 mm Hg systolic pressure),

tachycardia (8 bpm), and a 65% increase in muscle sympathetic nerve activity.²⁵ Lipid status can contribute to an elevation in blood pressure by altering endothelial function.²⁶ Moreover, a recent study in overweight humans with primary hypertension found that atorvastatin treatment resulted in a reduction in muscle nerve activity independent of blood pressure, indicating that the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase may activate sympathetic drive.²⁷ Irrespective of the mechanism underlying the phenomenon, this has implications for cardiovascular health in people who are constantly in a cycle of weight gain and acute dieting to reduce body weight. During the weight gain phase, MAP and RSNA rise rapidly, but even after successfully reducing caloric intake, these individuals may retain elevated RSNA and MAP.

Our model is one of acute fat feeding, and withdrawal of the HFD resulted in a rapid recovery of HR, which fell to baseline levels within 1 week. Interestingly, this fall in hemodynamic factors is not dependent on a fall in body weight or low body fat but may be more likely related to plasma insulin and glucose concentrations, which also return to baseline within 1 week of recovery from the HFD. These observations support the hypothesis that elevated hemodynamics occur under conditions of caloric load.

Stress and Obesity

A number of studies implicate increased cardiovascular responses to emotional stress as being pivotal in the development of hypertension.^{28,29} It is also reported that obese

humans demonstrate an exacerbated cardiovascular and sympathetic response to mental and cold pressor stressors.¹³ Our finding of invariant cardiovascular responses to air-jet stress between HFD and control animals suggests that, early in the development of obesity-related hypertension, there is no potentiation of stress pathways. In fact, we observed progressive diminution of blood pressure, HR, and RSNA responses to stress as the HFD diet progressed. This reduction in response is not habituation for MAP or HR at least, because the response to air-jet stress returned to baseline levels on withdrawal of the HFD. Although the mechanism underlying this reduction in stress responses is not known, one possible scenario is that the elevation in blood pressure, HR, and RSNA observed with HFD feeding is a result of activation of endogenous stress pathways, and, therefore, when these pathways are activated by the air-jet stressor, the net increase to maximal response is less. We have shown previously that the dorsomedial hypothalamus is pivotal in the transduction of the stress response.³⁰

Baroreflexes But Not Chemoreflexes Become Aberrant in Early Onset Obesity

Our data indicate that short-term HFD consumption results in alterations to the HR and RSNA baroreflexes. In the first and subsequent weeks of HFD intake, there was a reduction in the RSNA baroreflex gain and range brought about by an elevation of the lower plateau without any change in upper plateau. Interestingly, the baroreflex control of RSNA did not simply reset toward the higher level of BP, and given the reduction in the stress response with HFD, it appears that a feed-forward sympathoexcitatory mechanism involving activation of nonbaroreceptor-dependent RSNA may be responsible for the increase in RSNA rather than an impaired baroreflex.

The gain of the HR baroreflex was also reduced during HFD consumption but normalized on withdrawal of the HFD. This finding is in agreement with previous reports in humans and animals. The hypertension in obesity is characterized by a reduction in baroreceptor inhibition in humans³¹ when there is significant visceral adiposity but elevation in subcutaneous adiposity does not alter the baroreflex. Consistent with this, weight loss is associated with reactivation of the baroreflex,¹⁴ and activation of the baroreflex by exogenous electric stimulation of the carotid sinus baroreceptors in obese hypertensive dogs results in a reduction in MAP, HR, and plasma norepinephrine concentrations.³²

Despite finding alterations to baroreflex function in HFD rabbits, the MAP, HR, or RSNA response to hypoxia was not different from controls, indicating that the chemoreflex is not changed in the early phase of obesity-related hypertension. Given the well-established link among obesity, obstructive sleep apnea, and sympathetic activation,^{33,34} this observation was perhaps unexpected. Repeated episodes of hypoxia, either occurring as a result of obstructive sleep apnea³⁵ or because of experimental induction, both result in elevation of blood pressure³⁶ and altered metabolic function.³⁷ There is also a decrease in baroreceptor sensitivity in patients with long-term obstructive sleep apnea.³⁸ Our data indicate that altered chemoreflex activity is not an instigating factor for obesity-related hypertension.

Perspectives

We have now shown that RSNA increases in conscious, unrestrained animals as they develop obesity-related hypertension and provides further evidence that obesity-related hypertension is neurogenic. This phase of obesity-related hypertension is associated with alterations in the baroreflex, but stress and chemoreflex pathways do not appear to be involved. Normalization of caloric intake results in partial reversal of blood pressure even when body weight remains high, adding further weight to the hypothesis that sympathetic nervous system activation in obesity occurs to maintain energy homeostasis, and obesity-related hypertension is a negative adverse effect. Further studies aimed at understanding how this regional-specific sympathetic vasomotor tone is controlled may offer a useful management of obesity-related hypertension by allowing targeted reduction of RSNA while maintaining brown adipose tissue sympathetic nerve activity to increase thermogenesis.

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Disclosures

None.

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Novelty and Significance

What Is New?

- RSNA, HR, MAP, plasma insulin, and plasma leptin increase in the first week of a high-fat, high-calorie diet in conscious rabbits.
- Blood pressure and RSNA remain elevated when the diet is withdrawn, but insulin and leptin return to normal.
- Sympathetic baroreflexes are impaired, but stress and chemoreflex pathways are unchanged.

What Is Relevant?

- Elevated sympathetic drive and impaired baroreflex function appear integral to the rapid development of obesity-related hypertension. Even when the high-fat diet is withdrawn, RSNA remains high.

Summary

Elevated sympathetic drive appears to be important for obesity-related hypertension, but although increased plasma leptin and insulin may contribute to the initiation of hypertension, they are not required for its maintenance.

ON-LINE SUPPLEMENT

RAPID ONSET OF RENAL SYMPATHETIC NERVE ACTIVATION IN RABBITS FED A HIGH FAT DIET

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Effect of HFD on adiposity and body composition

Rabbits fed a HFD for 3 weeks followed by 1 week normal diet showed a 2-3 fold greater weight of perirenal, mesenteric, testicular and bladder WAT compared with control rabbits in absolute terms or when expressed as % body weight (all $P_{\text{group}} < 0.001$, Table S3). BAT weight in HFD rabbits was also more than double that of control rabbits ($P_{\text{group}} < 0.01$, Table 4). Although left ventricular weight was 11 % greater in rabbits previously fed a HFD than controls (HFD 4.6 g vs control 4.2 g, $P_{\text{group}} = 0.01$), this difference was abolished when weights were scaled to body weight. Interestingly, scaled weight of the liver was significantly less in HFD rabbits than controls (HFD 2.7 % vs control 3.1 %, $P_{\text{group}} = 0.01$). The weights of kidneys and adrenal glands were similar in both treatment groups.

In a separate group of rabbits, body composition was determined after 1 or 3 weeks on either a control or HFD (no return to control diet). Lean body mass and bone mineral content were not altered by 1 or 3 weeks of HFD. However, fat mass as a percentage of body weight was greater in HFD fed rabbits than control rabbits after 1 week of diet ($4.50 \pm 0.00\%$ vs $2.55 \pm 0.35\%$, respectively, $P < 0.05$). After 3 weeks HFD, fat mass ($6.93 \pm 0.98\%$) was markedly greater than at 1 week ($P < 0.05$). Lean muscle mass as percentage of body weight was reduced in 3 week HFD fed rabbits compared to controls ($P < 0.01$).

Table S1 Average resting values and renal sympathetic nerve activity baroreflex parameters

Control	Baseline	Week 1	Week 2	Week 3	SEM
MAP (mmHg)	71	68	67	69	2
RSNA (nu)	6.6	6.7	8.6	8.5	1.0
Lower plateau (nu)	4.9	5.1	6.1	6.1	0.7
Range (nu)	12.3	11.3	12.7	11.8	1.0
Upper plateau (nu)	17.2	16.4	18.8	17.9	1.4
BP50 (mmHg)	63	62	60	63	2
Gain (nu mmHg ⁻¹)	-1.06	-1.03	-0.92	-0.77	0.16
HFD					
MAP (mmHg)	72	71	78 *	74	2
RSNA (nu)	5.0	6.7	6.8	9.1 ‡	0.8
Lower plateau (nu)	3.6	5.2 *	5.6 †	7.0 ‡	0.5
Range (nu)	13.9	11.4 *	9.1 ‡	9.8 ‡	1.1
Upper plateau (nu)	17.5	16.6	14.7	16.8	1.3
BP50 (mmHg)	64	63	69 *	68	2
Gain (nu mmHg ⁻¹)	-1.08	-0.80	-0.64 †	-0.57 †	0.12

Values are mean and SEM indicating within animal variance in control rabbits (n = 7) and rabbits fed a high fat diet (HFD, n = 6) before and at weekly intervals after commencement of HFD. * P < 0.05, † P < 0.01, ‡ P < 0.001 for weekly value vs baseline. MAP, mean arterial pressure, RSNA, renal sympathetic nerve activity (normalized units), BP50, blood pressure at half the baroreflex range.

Table S2 Average resting values and heart rate baroreflex parameters

Control	Baseline	Week 1	Week 2	Week 3	Recovery	SEM
MAP (mmHg)	71	69	69	72	69	1
Heart rate (b min ⁻¹)	179	176	175	174	164	‡ 4
Lower plateau (b min ⁻¹)	134	127	132	125	129	5
Range (b min ⁻¹)	202	203	204	218	201	6
Upper plateau (b min ⁻¹)	336	330	336	343	330	6
BP50 (mmHg)	65	63	63	66	61	* 1
Gain (b/min mmHg ⁻¹)	-9.34	-8.42	-8.42	-10.00	-10.72	0.81
HFD						
MAP (mmHg)	71	74	76	† 77	‡ 73	2
Heart rate (b min ⁻¹)	187	198	† 199	† 200	† 173	‡ 4
Lower plateau (bmin ⁻¹)	142	155	* 145	146	128	* 6
Range (b min ⁻¹)	192	165	‡ 198	208	* 218	‡ 7
Upper plateau (b min ⁻¹)	334	320	* 343	354	† 346	6
BP50 (mmHg)	66	69	* 70	† 71	‡ 69	* 1
Gain (b/min mmHg ⁻¹)	-8.32	-6.64	* -6.70	* -7.20	-9.62	0.61

Values are mean and SEM indicating within animal variance in control rabbits (n = 10) and rabbits fed a high fat diet (HFD, n = 12) before and at weekly intervals after commencement of HFD and return to control diet (recovery). * P < 0.05, † P < 0.01, ‡ P < 0.001 for weekly value vs baseline. Abbreviations as for Table S1.

Table S3. Weight of fat pads from rabbits fed a control or high fat diet, taken one week after return to control diet

Adipose Tissue Depot	Control	HFD	
Mesenteric WAT (g)	24.9 ± 2.6	61.7 ± 4.6	†
Mesenteric WAT (% BW)	0.8 ± 0.1	1.7 ± 0.1	†
Perirenal WAT (g)	25.0 ± 2.7	66.5 ± 6.7	†
Perirenal WAT (% BW)	0.8 ± 0.1	1.9 ± 0.2	†
Test/blad WAT (g)	3.7 ± 0.5	7.3 ± 0.6	†
Test/blad WAT (% BW)	0.1 ± 0.0	0.2 ± 0.0	†
Brown adipose tissue (g)	5.9 ± 0.7	13.5 ± 1.6	*
Brown adipose tissue (%BW)	0.2 ± 0.0	0.4 ± 0.0	*
n	8	11	

Values are mean ± SEM, expressed as weight in grams and as a percentage of body weight (BW). * P < 0.01, † P < 0.001 for comparison of control with high fat diet (HFD). WAT, white adipose tissue; test/blad, testicular, bladder