Exposure to a High-Fat Diet During Development Alters Leptin and Ghrelin Sensitivity and Elevates Renal Sympathetic Nerve Activity and Arterial Pressure in Rabbits

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Abstract—Exposure to maternal obesity or a maternal diet rich in fat during development may have adverse outcomes in offspring, such as the development of obesity and hypertension. The present study examined the effect of a maternal high-fat diet (m-HFD) on offspring blood pressure and renal sympathetic nerve activity, responses to stress, and sensitivity to central administration of leptin and ghrelin. Offspring of New Zealand white rabbits fed a 13% HFD were slightly heavier than offspring from mothers fed a 4% maternal normal fat diet (P<0.05) but had 64% greater fat pad mass (P<0.015). Mean arterial pressure, heart rate, and renal sympathetic nerve activity at 4 months of age were 7%, 7%, and 24% greater, respectively (P<0.001), in m-HFD compared with maternal normal fat diet rabbits, and the renal sympathetic nerve activity response to airjet stress was enhanced in the m-HFD group. m-HFD offspring had markedly elevated pressor and renal sympathetic nerve activity responses to intracerebroventricular leptin (5–100 µg) and enhanced sympathetic responses to intracerebroventricular ghrelin (1–5 nmol). In contrast, there was resistance to the anorexic effects of intracerebroventricular leptin and less neuronal activation as detected by Fos immunohistochemistry in the arcuate (−57%; P<0.001) and paraventricular (−37%; P<0.05) nuclei of the hypothalamus in m-HFD offspring compared with maternal normal fat diet rabbits. We conclude that offspring from mothers consuming an HFD exhibit an adverse cardiovascular profile in adulthood because of altered central hypothalamic sensitivity to leptin and ghrelin. (Hypertension. 2014;63:338-345.)

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

With the rising prevalence of obesity, particularly among women of reproductive age, it is important to understand the consequences of maternal obesity and nutrient excess on processes underlying development because they may lead to adverse outcomes in offspring. Animal models of maternal fat-rich diets that reflect the dietary intakes of humans in affluent societies have demonstrated the programming of offspring hyperphagia, adiposity, insulin resistance, and hypertension.1,2 Various factors, including elevated blood pressure (BP), insulin resistance, hyperglycemia, elevated plasma glucocorticoids, and leptin, associated with obesity during gestation can affect the development of a fetus.3 These factors can all contribute to a suboptimal intrauterine environment and predispose offspring to an increased risk of obesity and hypertension. There is evidence that the sympathetic nervous system (SNS) may be activated in offspring of fat-fed dams that develop hypertension,1 but to date no study has shown direct evidence for increased sympathetic vasomotor activity. The adipokine hormone leptin and the gut hormone ghrelin act on neural circuitry of the hypothalamus important for energy homeostasis and the regulation of the SNS.4,5 We have recently shown that the renal sympathetic nerve activity (RSNA) and BP increases in the first few days of consuming a high-fat diet (HFD) in rabbits can be largely reversed by a leptin antagonist given intracerebroventricularly (ICV).6–9 In contrast, ghrelin secreted by the stomach activates arcuate neurons containing neuropeptide Y and agouti-related protein and has been shown to suppress sympathetic activity, decrease BP, and stimulate appetite.10,11 Thus, both leptin and ghrelin may play a key role in the association between obesity and hypertension and may also be involved in the hypertension programmed by a maternal HFD (m-HFD).

The purpose of this study was to examine the effect of an m-HFD during development on arterial pressure and RSNA and the central sympathetic effects of leptin and ghrelin in offspring. We used a rabbit model in which an HFD induces elevated BP and RSNA.6–9 We hypothesized that offspring exposed to an HFD during development would retain greater deposits of fat and show a selective leptin-resistant phenotype.
with altered cardiovascular and sympathetic responses to leptin and ghrelin.

Materials and Methods

Animals

Experiments were approved in advance by the Alfred Medical Research Education Precinct Animal Ethics Committee, and all procedures were conducted in accordance with the Australian Code of Practice for Scientific Use of Animals. Four proven female breeders (1 previous litter) and 2 male New Zealand white rabbits were meal fed a normal fat diet (NFD) and housed individually under controlled light (0600–1800 h) and temperature (22±2°C) conditions. Three weeks before mating, female rabbits were given ad libitum access to the NFD (4.2% fat, 2.63 kcal/g; GPR [meat free], Specialty Feeds, Glen Forrest, Australia) or an HFD (13.3% fat, 3.34 kcal/g; SF06-011, Specialty Feeds), and rabbits remained on these diets throughout gestation and lactation (see Figure 1 for timeline). Offspring from 3 maternal NFD (m-NFD) and 3 m-HFD pregnancies were used. This resulted in a total of 8 m-NFD (male=6, female=2) and 9 m-HFD offspring (male=6, female=3). After weaning at 8 weeks, rabbits were housed individually. They were meal fed a restricted NFD and were weighed weekly.

Experimental Procedures and Protocol

At 12 weeks of age (see Figure 1 for timeline), rabbits were anesthetized (isoflurane with carprofen 3 mg/kg analgesia before and 24 hours after surgery) and instrumented with an ICV cannula implanted into the lateral ventricle. At 14 weeks, an initial experiment was performed to assess food intake (provided ad libitum at regular daily feeding times) measured for 24 hours immediately after a single dose of either leptin (100 μg in 50 μL ICV) or vehicle (Ringer’s solution, 50 μL ICV) with 3 days between each measurement. At 16 weeks, a renal nerve recording electrode was implanted 6 to 7 days before experimentation. At 17 to 18 weeks, the main experiments were performed. Mean arterial pressure (MAP) and heart rate (HR) were measured via the central ear artery that was catheterized transcutaneously with a 22-gauge needle (Statham P23DG transducer; Hato Rey, Puerto Rico, Brazil). MAP, HR, and RSNA were recorded maximum nasopharyngeal response evoked by smoke at the beginning of each experiment.13 MAP, HR, and RSNA were recorded at baseline for 60 minutes in response to airjet stress (a stream of air directed at the rabbit’s nose at 60 L/min for 10 minutes) and after administration of leptin, ghrelin, and Ringer’s solution with 2±2 days between experiments. Parameters were measured for 30 minutes in response to ICV administration of leptin at increasing doses (5, 10, 50, and 100 μg; Pepro Tech Inc, NJ) and for 60 minutes after ICV administration of ghrelin at increasing doses (1, 2.5, and 5 nmol; Auspep, Victoria, Australia). The cardiovascular and RSNA responses to leptin and ghrelin were calculated as a change from vehicle (Ringer’s solution, 50 μL) administered before 30 minutes.

Tissue Collection

One week after cardiovascular parameters were measured, 18-week-old rabbits were deeply anesthetized with sodium pentobarbitone (100 mg/kg IV) and transcardially perfused with 1 L of phosphate buffer and 1 L of 4% paraformaldehyde. The liver, kidney, heart adrenal gland, retroperitoneal white adipose tissue (surrounding the kidney), and visceral white adipose tissue (surrounding the stomach and intestines) were dissected and weighed for comparison. Brains were removed from rabbits (n=5 per group) for Fos immunohistochemical analysis of neurons activated by a final injection of leptin (100 μg ICV).13,15

Data Analysis

Values are presented as mean±SEM and mean difference±SE of the difference. Data were analyzed by multifactor repeated-measures ANOVA for the main effect of diet using the sum of the between- and within-animal residual sums of squares as described previously.16 Intralitter variability was also included as part of the between-animal effect. For baseline measurements of MAP, HR, and RSNA, a within-animal contrast was made between 2 experimental days using the within-animal residual calculated by subtracting the between-animal and treatment (time) sums of squares from the total. For responses to leptin, ghrelin, and airjet stress, within-animal orthogonal contrasts were made in which the within-animal estimate of variance was calculated by combining the within-animal residual from both groups. The dose–response effect of leptin and ghrelin was calculated using a linear orthogonal contrast for ghrelin and the 10, 50, and 100 μg doses of leptin for each diet group. Single variables, such as fat pad mass, organ weights, and food intake responses to leptin, were analyzed using an independent Student t test. A P<0.05 was considered significant.

Results

Effect of m-HFD on Body Weight, Fat Pads, and Organ Weights

During the monitoring period from 9 to 18 weeks, all offspring steadily increased in body weight in a characteristic asymptotic growth curve and had gained an average of 53.2% in the m-HFD and 53.6% in the m-NFD groups. However, the m-HFD rabbits were always slightly heavier throughout the 4-month period (F between groups=16.3; P<0.001; Figure 2). At the end of the study, the postmortem analysis revealed that the combined retroperitoneal and visceral white adipose tissue fat pads were 64% heavier in rabbits from mothers fed an HFD (P=0.015; Table 1). Differences remained after adjustment for body weight (Table 1). Body organs, including the heart, liver, adrenal glands, and kidney, were similar between groups (Table 1).

Effect of m-HFD on Baseline Cardiovascular Variables and RSNA

Cardiovascular variables were elevated in offspring from mothers fed an HFD compared with offspring from mothers fed a normal diet (m-NFD) or high-fat diet (m-HFD). HR indicates heart rate; ICV, intracerebroventricularly; MAP, mean arterial pressure; and RSNA, renal sympathetic nerve activity.
fed an NFD at 4 months of age. MAP and HR were both 7% greater, whereas RSNA was elevated by 24% in the m-HFD group ($P<0.001$, Table 2). The higher RSNA was because of greater burst frequency (+22%, $P<0.01$) but not amplitude which was similar in the 2 groups (Table 2).

**Effect of m-HFD on Responses to Airjet Stress**

Exposure to airjet stress for 10 minutes increased MAP, HR, and RSNA in both m-NFD and m-HFD offspring (Figure 3). The changes in MAP and HR from baseline were similar in the 2 groups, but the rise in RSNA was 23% greater in the m-HFD offspring (3.5±0.2 versus 2.8±0.3 normalized units; $P<0.05$; Figure 3).

**Effect of ICV Administration of Leptin on Cardiovascular Variables and RSNA**

Administration of increasing doses of leptin ICV increased MAP (8%–9% at the highest dose of 100 μg) and HR (8% to 11%) in both m-NFD and m-HFD rabbits ($P<0.01$, Figure 4). However, the marked increase in RSNA (9% at the highest dose; $P<0.05$) only occurred in m-HFD offspring (Figure 4). m-HFD rabbits had greater MAP and RSNA responses to leptin than m-NFD rabbits ($P<0.05$; Figure 4). Injection of the vehicle Ringer’s solution did not alter MAP, HR, or RSNA (Figure 4).

**Effect of ICV Administration of Leptin on Food Intake**

ICV administration of a single dose of leptin (100 μg) reduced 24-hour food intake relative to the effect of Ringer’s solution to a greater extent in m-NFD rabbits compared with m-HFD rabbits (−21.3±4.6 versus −6.1±3.1 g, respectively; $P<0.05$). The baseline food intake was slightly higher in m-NFD rabbits compared with m-HFD rabbits (221±7 versus 205±5 g, respectively; $P=0.050$, df=13).

**Effect of ICV Administration of Leptin on Fos Immunoreactivity**

After a single ICV injection of leptin, less neuronal activation, as detected by Fos immunohistochemistry, was identified in several brain regions in m-HFD rabbits compared with m-NFD rabbits. The regions with less activation were the arcuate and paraventricular nuclei in the hypothalamus, the central and medial amygdala, and the medial preoptic nucleus and medial preoptic area (Figure 5). No differences were observed between groups in the supraoptic nucleus, the dorsomedial or ventromedial hypothalamus, the medial preoptic nucleus, the bed nucleus of the stria terminalis, or the hindbrain (Figure 5).
Effect of ICV Administration of Ghrelin on Cardiovascular Variables and RSNA

ICV administration of ghrelin produced similar dose-dependent falls in MAP (7%–10% at the highest dose of 5 nmol) and HR (21% to 24%) in m-NFD and m-HFD rabbits (P<0.05; Figure 6). In contrast, ghrelin elevated RSNA in m-HFD rabbits (+13% over doses; P<0.01) but not in m-NFD rabbits (Figure 6). Injection of Ringer’s solution did not alter MAP, HR, or RSNA (Figure 6).

Discussion

The major findings of the current study were that MAP, HR, and RSNA were elevated in offspring from mothers fed an HFD compared with offspring from mothers fed an NFD at 4 months of age. Furthermore, m-HFD rabbits had markedly elevated RSNA and pressor responses to central leptin and enhanced sympathetic responses to ghrelin. In contrast, the appetite-suppressant effect of leptin was diminished in m-HFD offspring suggesting a selective leptin-resistant phenotype, whereby m-HFD offspring are resistant to the appetite-suppressant effects of leptin but hyper-responsive to the stimulatory cardiovascular effects of leptin. We also observed an exaggerated sympathetic response to acute airjet stress, suggesting that the higher levels of BP and RSNA may be attributable to changes in several central pathways regulating the SNS. We have previously demonstrated that an HFD given to adult rabbits produces a similar exaggerated response to leptin and that a leptin antagonist given ICV reverses the high BP and elevated RSNA.8,9 Taken together, these findings suggest that a specific change in the sympathetic response to leptin may contribute to the elevated RSNA and BP observed in m-HFD offspring.

An elevated level of leptin would be expected because of the higher levels of visceral fat in the m-HFD offspring. However, it is difficult to discern whether the elevated cardiovascular and sympathetic variables in m-HFD rabbits are a result of the excess accumulation of visceral fat or because of programming of the SNS by an early nutritional effect of an HFD. The fat deposited in the offspring (46 g) is approximately half the amount accumulated during 3 weeks of an HFD in adult rabbits (92 g) that produced a similar phenotype of higher BP and RSNA and an exaggerated response to ICV leptin.8,9 At present, we do not know whether these effects seen at 18 weeks persist. White et al17 suggested that excess maternal body fat is the primary mechanism driving obesity.
in offspring. However, Rudyk et al.\textsuperscript{18} proposed that a fat-rich maternal diet with no maternal obesity was responsible for an exaggerated response to stress and hypertension in adult offspring. Although short-term exposure to an HFD in the absence of maternal obesity has not been shown to be associated with elevated resting BP in offspring,\textsuperscript{18} BP is elevated in the offspring of obese rats.\textsuperscript{19} An earlier study in rabbits has indicated that maternal BP is critical in determining offspring BP, where female offspring of mothers with secondary renal hypertension had elevated BP.\textsuperscript{20} Hence, elevated BP in mothers fed an HFD may be a factor programming the elevated BP and RSNA in offspring. In the current study, breeding female rabbits were fed an HFD for \( \geq 3 \) weeks before mating, and as observed in our earlier studies,\textsuperscript{7–9} a 3-week feeding regime would be expected to raise adipose tissue, BP, HR, and RSNA in mothers, which may all contribute to the offspring phenotype.

Our study is novel in that we show the developmental programming of selective leptin resistance in offspring from mothers fed an HFD with regard to not only reduced satiety but also increased RSNA and BP in response to central administration of leptin. The concept of selective leptin resistance associated with obesity has been described in mouse models that were obese because of genetic defects\textsuperscript{21} or after an HFD in mice\textsuperscript{22,23} or rabbits.\textsuperscript{8,9} The current study differs in that rabbits were only exposed to overnutrition during development and were thereafter fed a restricted NFD after weaning. Hence, elevated BP in mothers fed an HFD may be a factor programming the elevated BP and RSNA in offspring. In the current study, breeding female rabbits were fed an HFD for \( \geq 3 \) weeks before mating, and as observed in our earlier studies,\textsuperscript{7–9} a 3-week feeding regime would be expected to raise adipose tissue, BP, HR, and RSNA in mothers, which may all contribute to the offspring phenotype.
and RSNA responses to leptin in these animals. The pattern closely resembled that observed in adult rabbits fed an HFD. Possible mechanisms include reduced hypothalamic STAT-3 (signal transducer and activator of transcription 3) phosphorylation or increased levels of the orexigenic neuropeptide Y in offspring, together with reduced levels of POMC (pro-opiomelanocortin). Hence, alterations in the levels of these neuropeptides may affect appetite and sympathetic activation in offspring from mothers fed an HFD and may explain the greater pressor and sympathetic responses to leptin observed in m-HFD rabbits compared with m-NFD rabbits.

Ghrelin may also play a role in the development of hypothalamic circuits and acts on similar hypothalamic leptin pathways to regulate appetite and sympathetic activity. In the current study, ghrelin dose dependently reduced MAP and HR similarly in both m-HFD and m-NFD animals, consistent with the findings of previous research in rabbits. However, unlike the finding of Matsumura et al., central administration of ghrelin did not reduce RSNA. Ghrelin has also been reported to increase muscle sympathetic activity in both lean and obese men. In the current study, offspring from mothers fed an NFD showed no change in RSNA, whereas m-HFD rabbits had elevated RSNA in response to ghrelin. This elevation occurred at the low dose of ghrelin that produced little change in MAP or HR; hence, renal sympathetic outflow is not likely to be the primary mechanism by which ghrelin at higher doses reduces MAP and HR. It is possible that ghrelin reduced MAP centrally by reducing sympathetic nerve activity to the heart or to nonrenal vasculature. Previous research has shown that in conscious rats with myocardial infarction, acute ghrelin administration reduces the elevated plasma concentration of norepinephrine to normal levels. Ghrelin has also been shown to reduce brown adipose tissue sympathetic nerve activity, although the effect of ghrelin on sympathetic activity to the vasculature has not previously been observed. Another possible mechanism by which ghrelin acts to reduce BP is by reducing cardiac output and HR, possibly via changes to the baroreceptor reflex. Ghrelin at subpressor doses has been shown to reduce baroreflex sensitivity. Hence, ghrelin might not only inhibit the SNS but may also activate the parasympathetic nervous system, which could explain the bradycardia observed after ghrelin administration. Future experiments with selective cardiac vagal or sympathetic blockade could readily resolve this question.

RSNA responses to an acute stress were examined to determine whether sympathetic pathways were affected by the maternal HFD. In the current study, the RSNA response to 10 minutes of airjet stress was markedly greater in the offspring from mothers fed an HFD, but pressor and tachycardic responses were similar in the 2 groups. The exaggerated RSNA activation in m-HFD offspring suggests that the pathways within the central nervous system mediating the sympathetic responses to stress are altered by the early exposure to a maternal HFD. Rudyk et al. showed exaggerated

Figure 6. Effect of ghrelin. Left, Mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA, normalized units) at baseline and after intracerebroventricular (ICV) administration of vehicle (Ringer’s solution, 50 μL) in rabbit offspring from mothers fed a normal fat diet (m-NFD; open bars, n=8) or high-fat diet (m-HFD; closed bars, n=9). **P<0.01 for comparison between groups (m-NFD vs m-HFD). Right, Sixty-minute averages of the changes in MAP, HR and RSNA compared with vehicle (left) after ICV administration of ghrelin to m-NFD (open) and m-HFD (closed) offspring. The gray line represents a vehicle time control averaged from both groups. Values are mean±SEM and mean differences±SE of the difference indicating variance between rabbits. (*)P=0.06 for comparison between groups (m-NFD vs m-HFD); #P<0.05, ##P<0.01, ###P<0.001 for comparison of ghrelin with vehicle.
pressor responses to stress in rat offspring but did not directly measure sympathetic nerve activity. Furthermore, the higher levels of MAP and HR achieved during the control and stress period in m-HFD offspring would suggest a greater cardiovascular risk in these offspring if these findings translated to humans exposed to a stressful lifestyle. A further consideration is that the greater pressor and sympathetic responses to leptin and ghrelin were not because of a general amplification of sympathetic pathways but were the result of specific adaptations in the leptin and ghrelin signaling pathways during development. Because adverse nutritional levels during fetal and neonatal life have been shown to alter the development of the hypothalamic–pituitary–adrenal axis, hormonal or adrenal responses to longer periods of stress or even chronic stress may be altered but were not assessed in the current study.

Perspectives
This study highlights how maternal nutrition can adversely affect cardiovascular risk factors in the next generation and provides a basis for understanding the effect of overnutrition during prenatal development on the central pathways involved in obesity-related hypertension. These findings suggest that a specific change in the sympathetic response to leptin and the elevated visceral fat accumulation observed in these offspring may contribute to their elevated BP, RSNA, and response to acute stress. The important question will be to determine the central sites and mechanisms leading to the transgenerational effects of a maternal HFD on the prohypertensive actions of leptin.

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Disclosures
None.

References
What Is New?

- Offspring from mothers fed a high-fat diet (HFD) at 4 months of age have elevated blood pressure, heart rate, and renal sympathetic nerve activity compared with offspring from mothers fed a normal fat diet.
- Offspring from mothers fed an HFD also have a greater increase in renal sympathetic nerve activity to central leptin and enhanced sympathetic responses to ghrelin.
- The appetite-suppressant effect of leptin was diminished in offspring from mothers fed an HFD.

What Is Relevant?

- Offspring from mothers consuming an HFD exhibit an adverse cardiovascular profile in adulthood because of altered central sensitivity to leptin and ghrelin.

### Novelty and Significance

**What Is New?**

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**What Is Relevant?**

- Offspring from mothers consuming an HFD exhibit an adverse cardiovascular profile in adulthood because of altered central sensitivity to leptin and ghrelin.

**Summary**

Rabbit offspring from dams fed a high-fat diet during the fetal development and suckling periods but a normal diet from then on have higher blood pressure, heart rate, and renal sympathetic nerve activity at 4 months of age. Offspring also show enhanced sympathoexcitatory effects of central doses of leptin but resistance to its appetite-suppressing actions.
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