

ORIGINAL RESEARCH

Loss of the Protective Effect of Estrogen Contributes to Maternal Gestational Hypertension-Induced Hypertensive Response Sensitization Elicited by Postweaning High-Fat Diet in Female Offspring

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BACKGROUND: A recent study conducted in male offspring demonstrated that maternal gestational hypertension (MHT) induces hypertensive response sensitization (HTRS) elicited by postweaning high-fat diet (HFD). In this study, we investigated the sensitizing effect of MHT on postweaning HFD-induced hypertensive response in female rat offspring and assessed the protective role of estrogen in HTRS.

METHODS AND RESULTS: The results showed that MHT also induced a sensitized HFD-elicited hypertensive response in intact female offspring. However, compared with male offspring, this MHT-induced HTRS was sex specific in that intact female offspring exhibited an attenuated increase in blood pressure. Ovariectomy significantly enhanced the HFD-induced increase in blood pressure and the pressor response to centrally administered angiotensin II or tumor necrosis factor- α in offspring of normotensive dams, which was accompanied by elevated centrally driven sympathetic activity, upregulated mRNA expression of prohypertensive components, and downregulated expression of antihypertensive components in the hypothalamic paraventricular nucleus. However, when compared with HFD-fed ovariectomized offspring of normotensive dams, the MHT-induced HTRS and pressor responses to centrally administered angiotensin II or tumor necrosis factor- α in HFD-fed intact offspring of MHT dams were not potentiated by ovariectomy, but the blood pressure and elicited pressor responses as well as central sympathetic tone remained higher.

CONCLUSIONS: The results indicate that in adult female offspring MHT induced HTRS elicited by HFD. Estrogen normally plays a protective role in antagonizing HFD prohypertensive effects, and MHT compromises this normal protective action of estrogen by augmenting brain reactivity and centrally driven sympathetic activity.

Key Words: autonomic functions ■ blood pressure ■ estrogen ■ high-fat diet ■ maternal gestational hypertension

Sex-specific effects are widely observed in studies of prenatal programming of offspring cardiovascular function.^{1,2} Such studies have investigated the effects of maternal undernutrition, maternal

hypertension/preeclampsia, placental insufficiency, high dietary salt, high-fat diet, hypoxia, glucocorticoid, and nicotine exposure.^{3–10} In general, in female offspring, estrogen plays a protective role in the timing of

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CLINICAL PERSPECTIVE

What Is New?

- These studies demonstrate that maternal gestational hypertension sensitizes the postweaning high-fat diet–elicited hypertensive response in adult intact female offspring.
- Ovariectomy uncovered the maternal gestational hypertension–induced programming of enhanced blood pressure response to postweaning high-fat diet in female offspring of normotensive dams; this sensitization process is associated with enhanced pressor responses to central pressor agents.
- The data indicate that the sensitization of hypertensive response to postweaning high-fat diet is attributable to impairment of female sex hormone protective effects produced by maternal gestational hypertension in female offspring.

What Are the Clinical Implications?

- These findings help us understand the central protective mechanisms of female sex hormones in antagonizing sensitized obesity-related hypertension and suggest that different strategies are required for treating hypertension in men versus women.

Nonstandard Abbreviations and Acronyms

ANG II	angiotensin II
AT2-R	angiotensin II type 2 receptor
ER	estrogen receptor
HFD	high-fat diet
HR	heart rate
HTRS	hypertensive response sensitization
LFD	low-lard-fat diet
MHT	maternal gestational hypertension
PIC	proinflammatory cytokine
PVN	hypothalamic paraventricular nucleus
RAS	renin-angiotensin system
SNS	sympathetic nervous system

the onset and severity of hypertension programmed by prenatal insults, whereas testosterone contributes to increased blood pressure (BP) and cardiovascular risk in male offspring exposed to a developmental challenge in young adulthood.^{7–10} Either long-term estrogen supplementation or androgen receptor blockade prevents the increase in BP in aged female intrauterine growth-restricted offspring, suggesting a lost protective effect of estrogen or a shift in the testosterone/estrogen ratio in these offspring.^{11,12} In the offspring of

maternal gestational hypertension (MHT) model, we demonstrated that castration did not alter the sensitized hypertensive response to angiotensin II (ANG II) in adult male offspring, whereas ovariectomy uncovered MHT-induced programming of an enhanced BP response to ANG II in female offspring. Estrogen replacement only partially abrogated the MHT-augmented response to ANG II in female offspring.¹⁰ These results indicate that the regulatory effects of sex hormones on prenatal insult-induced developmental programming of the hypertensive response are related to experimental animal model and the age of offspring.

Obesity/high-fat diet (HFD) is a risk factor for cardiovascular diseases, including hypertension. There is compelling evidence that the cause of obesity-related hypertension is primarily through neurogenic mechanisms, which are characterized by activation of the sympathetic nervous system (SNS).^{13–15} However, the SNS is differently affected in men and women by sex hormones, body mass index, BP, and leptin levels, in which muscle sympathetic nervous activity mainly relates to BP in women and to body mass index in men.^{13,15–17} Some studies have shown that females lose cardiovascular protection from female sex hormones in obesity, suggesting that the protective effect of estrogen has been altered under obese/HFD conditions in females.¹⁶

It has been shown that perinatal programmed offspring can be affected by a “second hit,” such as HFD that exacerbates their cardiometabolic health in a sex-specific manner.^{18–23} In these studies, the researchers focused on peripheral mechanisms, such as renal function, glucose homeostatic response, and systemic and adipose renin-angiotensin system (RAS), that are involved in the sex differences in cardiometabolic dysfunction in offspring with both prenatal insult and postnatal HFD feeding. Although a large body of studies have shown central interactions between sex hormones and the RAS, proinflammatory cytokines (PICs), and leptin influencing SNS activity and BP,^{15,24–29} the mechanisms underlying central regulatory effects of sex hormones, especially estrogen regulation of autonomic function in HFD fed offspring of maternal hypertensive dams, have not been explored.

Our previous study indicates that MHT induces hypertensive response sensitization (HTRS) elicited by postweaning HFD through a mechanism of exaggerated centrally driven sympathetic activity and the enhanced pressor responses to central leptin and RAS or PIC components in male offspring.³⁰ In the current study, we investigated if the maternal hypertension also induces HTRS elicited by postweaning HFD in female offspring. Furthermore, we determined if estrogen plays a protective role in MHT-induced HTRS, and if estrogen protection is associated with regulation of brain reactivity to pressor agents and altered autonomic function in postweaning HFD fed female offspring.

METHODS

We will make all data related to the findings described in our article fully available from the corresponding authors upon reasonable request.

Animals

All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the University of Iowa Animal Care and Use Committee.

A total of 48 female and 48 male rats (Sprague-Dawley; 10 weeks old; Envigo) were used for breeding. All animals were maintained in a temperature (23 ± 2 °C) and light (12-hour light/dark cycle) controlled facility. Half of the females were chronically treated with vehicle (saline) and considered as normotensive dams, whereas the other half were treated with ANG II (SC; 250 ng/kg per minute; model 2004; 4 weeks; Alzet) throughout mating and pregnancy and considered as hypertensive dams. This dose of ANG II infusion induced a significant increase in BP during pregnancy (38.5 ± 6.2 mm Hg) in dams, as described in our previous study.³¹ The offspring were weighed and counted at birth, and the litter sizes reduced at 3 days of age to 4 male and 4 female pups. All offspring were weaned at 3 weeks of age, and feeding began with one half of the animals receiving a low-lard-fat diet (LFD; 10% calories from lard; 3.85 kcal/g; D12450J; Research Diets Inc) and the other one-half receiving a HFD (60% calories from lard; 5.24 kcal/g; D12492; Research Diets Inc) for 12 weeks. Male data from our studies have been published previously,³⁰ so that the results from female offspring are the focus of this report. At 8 weeks of age, half of the female offspring fed either LFD or HFD were ovariectomized, thereby yielding 8 groups: (1) normotensive dam+LFD-offspring, (2) normotensive dam+HFD-offspring, (3) hypertensive dam+LFD-offspring, (4) hypertensive dam+HFD-offspring, (5) normotensive dam+LFD-ovariectomized offspring, (6) normotensive dam+HFD-ovariectomized offspring, (7) hypertensive dam+LFD-ovariectomized offspring, and (8) hypertensive dam+HFD-ovariectomized offspring. Each experimental group was composed of individual subjects that were randomly selected from different litters. A total of 84 female offspring of normotensive dams and same number of female offspring of hypertensive dams were used in the present experiments. Food and body weight were weighed 1 time per week until the experiments began.

Figure 1 shows the timeline of the study design. Experiment 1: At 16 to 18 weeks of age, all groups of offspring were used to evaluate basal BP and heart rate (HR) using implanted telemetric probes ($n=5-6$

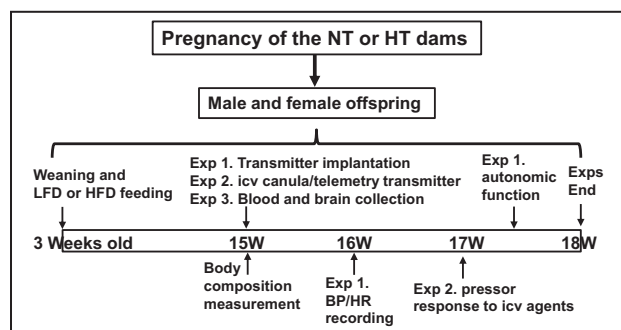


Figure 1. Representative timeline of the study design. Female offspring of normotensive (NT) or hypertensive (HT) dams were weaned at 3 weeks of age and fed with a low-lard-fat diet (LFD) or high-fat diet (HFD) until experiments ended when the animals were 18 weeks old.

Experiment 1 (Exp. 1): female offspring were instrumented with the telemetry transmitters after 12 weeks of dietary treatment, and hemodynamic and autonomic functions were measured. Experiment 2 (Exp. 2): intracerebroventricular (icv) cannulas and telemetry transmitters were implanted for assessment of pressor response to icv agents. Experiment 3 (Exp. 3): at 15 weeks of age, LFD or HFD fed female offspring from either NT or HT dams were euthanized to collect blood and brain tissues for assessing plasma levels and mRNA expression of renin-angiotensin system components, cytokines, or leptin. BP indicates blood pressure; and HR, heart rate.

per group). BP was also measured in the presence of the ganglionic blocker hexamethonium (30 mg/kg; IP), the muscarinic receptor blocker atropine (8 mg/kg; IP), or the β -adrenergic receptor blocker atenolol (8 mg/kg; IP) ($n=5-6$ per group). Experiment 2: In separate groups of offspring, pressor responses to intracerebroventricular injections of ANG II (200 ng/2 μ L), tumor necrosis factor- α (TNF- α ; 100 ng/2 μ L), or leptin (5 μ g/2 μ L) were determined through implantation of telemetry transmitters and brain lateral ventricle cannulas ($n=5-6$ per group). Experiment 3: The blood and brains from separate groups of offspring with the diet treatment were collected for analyses of plasma levels of ANG II, interleukin 6 (IL-6), and leptin ($n=6-9$ per group) and mRNA expression of the RAS components, PICs, and microglial marker ($n=5$ per group), respectively. The hypothalamic paraventricular nucleus (PVN) was used for these analyses.

Body Composition Measurement, Tissue Collection, and Blood Plasma Analysis

After 12 weeks of diet treatment, body composition, including total body fat, lean, and fluid masses, was determined by nuclear magnetic resonance spectroscopy using a Bruker mini-spec LF 90II instrument (Bruker Corporation, Billerica, MA). To analyze body composition, rats were placed into a restraint tube and inserted into the rodent-sized nuclear magnetic

resonance apparatus, adjusting the volume of the chamber based on the size of the animal.

After decapitation, trunk blood from all offspring was collected for biochemical assays. Plasma levels of ANG II (catalog No. CEA005Ra; Cloud Clone, Wuhan, China), interleukin 6 (IL-6) (catalog No. R6000B; R&D Systems, Minneapolis, MN), and leptin (catalog No. MOB00; R&D Systems) were measured with commercial ELISA kits, according to the manufacturers' instructions.

At same time, brains were collected for analysis of mRNA expression by real time polymerase chain reaction (PCR).

Telemetry Probe Implantations and Measurement of BP and HR

Rat telemetric probes (HD-S10; Data Sciences International, St. Paul, MN) were used to directly measure arterial pressure and HR in individual animals. At 14 weeks of age, all offspring were anesthetized with a ketamine-xylazine mixture (90% ketamine and 10% xylazine; IP), and the femoral artery was accessed with a ventral incision. The right femoral artery was isolated, and the catheter of a telemetric probe was inserted into the vessel. Through the same ventral incision, a pocket along the right flank was formed. The body of the transmitter was slipped into the pocket and secured with tissue adhesive. The ventral incision was then closed with suture. All rats were allowed 7 days to recover from transmitter implantation surgery. Thereafter, BP and HR were telemetrically recorded and stored with the Dataquest ART data acquisition system (Data Sciences International).

Evaluation of Autonomic Function

BP and HR were measured in the presence of the ganglionic blocker hexamethonium (30 mg/kg; IP), the muscarinic receptor blocker atropine (8 mg/kg; IP), or the β -adrenergic receptor blocker atenolol (8 mg/kg; IP) on 3 separate days, respectively. On the day of experiment, rats were allowed to stabilize for at least 60 minutes, after which time BP and HR were recorded for 20 to 60 minutes before and after administration of autonomic antagonists.

Intracerebroventricular Cannula Implantation and Evaluation of the Effects of Short-Term Microinjection of ANG II, TNF- α , or Leptin

At 14 weeks of age, offspring were anesthetized intraperitoneally with 90% ketamine and 10% xylazine, and rat telemetric probes (HD-S10; Data Sciences International) were implanted to measure arterial pressure and HR in individual offspring. At same time,

intracerebroventricular cannulas (25 gauge) were implanted into right lateral cerebral ventricle (the coordinates 1.0 mm caudal, 1.5 mm lateral to bregma, and 4.5 mm below the skull surface) for short-term bolus microinjections of vehicle (saline, 2 μ L; ANG II, 200 ng/2 μ L; TNF- α , 100 ng/2 μ L; or leptin, 5 μ g/2 μ L) delivered through 33-gauge injection cannulas. After 1 week of recovery, the effects of intracerebroventricular injections of ANG II, TNF- α , or leptin on BP and HR were determined in conscious intact and ovariectomized offspring.

Real-Time PCR Analysis

The intact and ovariectomized offspring with dietary treatments were decapitated, and the brains were quickly removed and put in iced saline for 1 minute. Then, the brain was cut into 200- μ m coronal sections, and the target tissues, the PVN, were punched with a 15-gauge needle stub (inner diameter, 1.5 mm). Some immediately surrounding tissue was usually included in the punch biopsies. The PVN is composed of magnocellular and parvocellular subregions. We collected all of the PVN for determination of mRNA expression. Total RNA was isolated from the PVN using the Trizol method (Invitrogen) and treated with DNase I (Invitrogen). RNA integrity was checked by gel electrophoresis. Total RNA was reverse transcribed using random hexamers following the manufacturer's instructions (Applied Biosystems). Real-time PCR was conducted using 200 to 300 ng of cDNA and 500 nmol/L of each primer in a 20- μ L reaction with iQ SYBR Green Supermix (Bio-Rad). Amplification cycles were conducted at 95 $^{\circ}$ C for 3 minutes, followed by 40 cycles of 95 $^{\circ}$ C for 15 seconds and annealing/extension at 60 $^{\circ}$ C for 30 seconds. Reactions were performed in duplicate and analyzed using a C1000 thermocycler system (Bio-Rad). mRNA levels for RAS components (angiotensin-converting enzyme 1 [ACE], angiotensin II type 1 receptor, angiotensin II type 2 receptor [AT2-R], and angiotensin-[1-7] receptor [Mas-R]), PICs (TNF- α , IL-6, and interleukin 10 [IL-10]), microglial marker (cluster of differentiation molecule 11b [CD11b]), leptin, and GAPDH were analyzed with SYBR Green real-time RT-PCR. The values were corrected by GAPDH, and the final concentration of mRNA was calculated using the formula $x=2^{(-\Delta\Delta Ct)}$, where x =fold difference relative to control. Primers were purchased from Integrated DNA Technologies (Coralville, IA). The sequences of the primers are shown in Table 1.

Statistical Analysis

Mean arterial pressure (MAP) and HR, obtained from the 10 days of telemetry recordings, are presented as mean daily values and averaged daily values of 10 days of recordings. Differences for BP were

Table 1. Primer Sequences for Real-Time PCR

Gene	Forward primer	Reverse primer	Product size, bp
<i>GAPDH</i>	TGACTCTACCCACGGCAAGTTCAA	ACGACATACTCAGCACCAGCATCA	141
<i>ACE</i>	GTGTTGTGGAACGAATACGC	CCTTCTTTATGATCCGCTTGA	187
<i>AT1-R</i>	CTCAAGCCTGTCTACGAAAATGAG	GTGAATGGTCCTTTGGTCGT	188
<i>AT2-R</i>	ACCTTTTGAACATGGTGCTTTG	TTTCTATGCCAGTGTGCAG	160
<i>Mas-R</i>	TGTGGTGGCTTTTCGATT	CCCGTCACATATGGAAGCAT	159
<i>TNF-α</i>	GCCGATTTGCCACTTCATAC	AAGTAGACCTGCCCGGACTC	209
<i>IL-6</i>	GCCTATTGAAAATCTGCTCTGG	GGAAGTTGGGGTAGGAAGGA	160
<i>IL-10</i>	CCTGCTCTACTGGCTGGAG	TGTCCAGCTGGTCCTTCTTT	178
<i>CD11b</i>	TTACCGGACTGTGTGGACAA	AGTCTCCCACCACCAAAGTG	239
<i>Leptin</i>	CCAAAACCCTCATCAAGACC	GTCCAAGTGTGAAGAATGTCCC	154

ACE indicates angiotensin-converting enzyme 1; AT1-R, angiotensin II type 1 receptor; AT2-R, angiotensin II type 2 receptor; CD11b, cluster of differentiation molecule 11b; IL-6, interleukin 6; IL-10, interleukin 10; Mas-R, angiotensin-(1-7) receptor; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor-α.

calculated for each animal based on the baseline subtracted from the BP after IP injection of hexamethonium or intracerebroventricular microinjection of ANG II (5 minutes), TNF-α (30 minutes), and leptin (30 minutes). Likewise, HR differences were calculated for each animal based on the baseline subtracted from the HR after IP injection of atenolol or atropine. All data were checked for the normality assumption and the homogeneity of variance by using Shapiro-Wilk test and Levene test, respectively. The 1-way or 2-way ANOVA was conducted on the means of calculated differences for each of the experimental groups. Post hoc analyses were performed with Tukey multiple comparison tests (equal variance) or Dunnett T3 tests (unequal variances, Brown-Forsythe and Welch ANOVA tests) between pairs of mean changes (Graph-Pad Prism 9.0). The same statistical methods were used to analyze the differences in metabolic parameters, and in plasma levels and mRNA expression of the RAS components, PICs, and leptin in the trunk blood and brain regions, respectively. All data are expressed as means±SEM. Statistical significance was set at $P<0.05$.

RESULTS

Effect of MHT and HFD Feeding on Metabolic Parameters in Intact and Ovariectomized Female Offspring

In intact female offspring, either MHT or postweaning HFD feeding resulted in significant increases in body weight when compared with LFD offspring of normotensive dams ($P<0.05$). Ovariectomy eliminated the MHT-produced difference in body weight gain between LFD fed offspring of normotensive and hypertensive dams ($P<0.05$) and resulted in a significant increase in body weight when compared with

the corresponding group of intact offspring ($P<0.05$). Moreover, HFD-produced body weight gain in ovariectomized offspring of normotensive dam ($P<0.05$), but not of hypertensive dam ($P>0.05$), was greater than that in LFD fed ovariectomized offspring of both normotensive and hypertensive dam ($F[7, 35]=25.35$; $P<0.0001$) (Figure 2 and Table 2).

In intact offspring, food intake (g/d) was greater in the offspring from both normotensive and hypertensive dams eating the LFD than those eating the HFD ($P<0.05$; Figure 3A). However, caloric intakes (calories/d) were similar among all groups of offspring ($P>0.05$; Figure 3B). As a result, feed efficiency was higher in LFD offspring of hypertensive dams and all HFD offspring when compared with LFD offspring of normotensive dams ($P<0.05$; Figure 3C and Table 2).

Ovariectomy resulted in a significant increase in food intake (g/d) in LFD-fed offspring, but not in HFD-fed offspring from both normotensive and hypertensive dams ($P<0.05$; Figure 3A). However, caloric intakes (calories/d) were similar among groups of ovariectomized offspring ($P>0.05$), and the caloric intakes in all ovariectomized offspring were only more than that in LFD-fed offspring of normotensive dam ($P<0.05$), but not in other groups of intact offspring (Figure 3B). Feed efficiency was higher in all groups of ovariectomized offspring when compared with corresponding intact offspring, and the feed efficiency in HFD fed ovariectomized offspring of both normotensive and hypertensive dams was greater than that in intact and ovariectomized offspring fed with LFD ($P<0.05$; Figure 3C and Table 2). Data are as follows (food intake: $F[7, 35]=44.73$; $P<0.0001$; caloric intake: $F[7, 35]=5.577$; $P<0.0002$; feed efficiency: $F[7, 35]=46.14$; $P<0.0001$).

Total adipose tissue mass was significantly increased after 12 weeks of HFD feeding in both intact and ovariectomized offspring from both normotensive and hypertensive dams when compared with

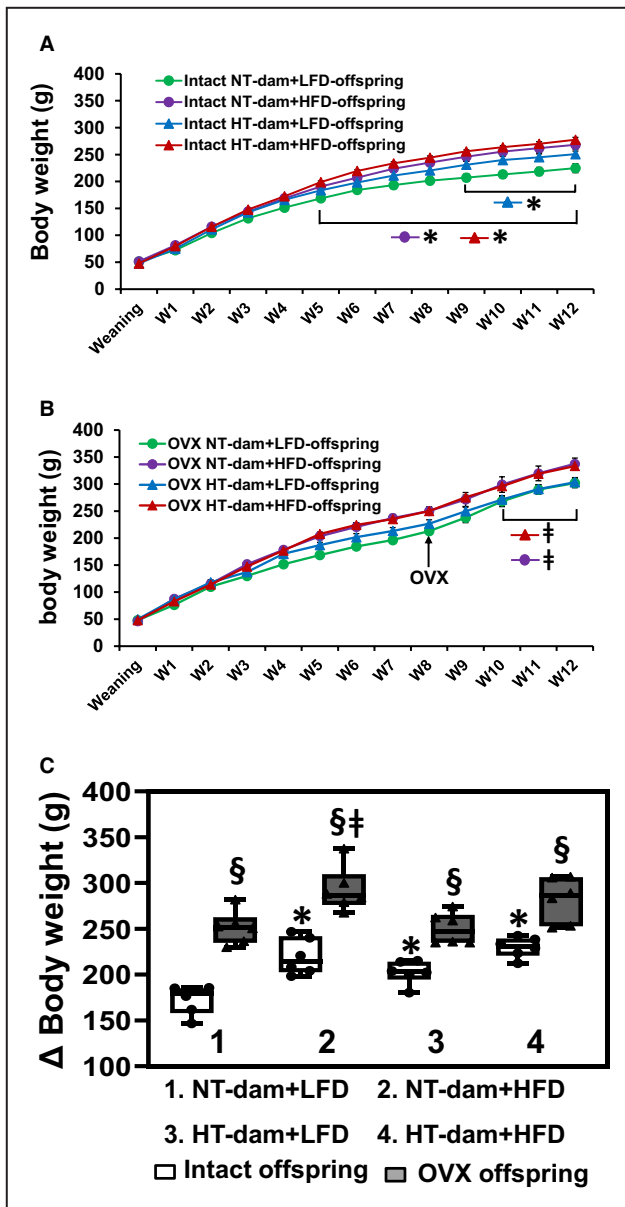


Figure 2. Increases in body weights in intact (A and C) and ovariectomized (OVX; B and C) female offspring from normotensive (NT) dams and hypertensive (HT) dams after 12 weeks (W) low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

Data are expressed as means±SEM, n=6/group; 2-way ANOVA was used for analysis followed by Tukey post hoc tests. * $P < 0.05$ vs NT-LFD offspring; † $P < 0.05$ vs intact or OVX LFD offspring; § $P < 0.05$ vs corresponding intact offspring.

those with LFD ($P < 0.05$; Figure 4A). Accordingly, fat contributed to a larger portion of total body components in HFD fed offspring ($P < 0.05$; Figure 4B). Ovariectomy significantly increased fat mass and fat composition only in HFD-fed offspring of normotensive dam, and these 2 fat parameters were kept higher in HFD-fed ovariectomized offspring of hypertensive dam when compared with LFD-fed intact

and ovariectomized offspring ($P < 0.05$; Figure 4A and 4B and Table 2). Data are as follows (Fat mass: $F[7, 69]=18.05$; $P < 0.0001$; fat composition: $F[7, 66]=13.15$; $P < 0.0001$).

Effect of MHT on Changes in BP and HR Induced by Postweaning HFD in Intact and Ovariectomized Offspring

HFD feeding significantly elevated basal MAP in intact female offspring from either normotensive or hypertensive dams when compared with LFD feeding (107.9 ± 0.9 versus 107.3 ± 0.8 mm Hg). However, the increases in MAP were greater in female offspring of hypertensive dams (121.3 ± 1.3 mm Hg) than that in female offspring of normotensive dams (115.2 ± 0.7 mm Hg) ($P < 0.05$; Figure 5A and 5C). In our previous study, male offspring were studied under identical experimental conditions to those described for the female offspring in the current study.³⁰ For the sake of comparison, the data from the previous report in males are compared with females in Table 3. When compared with the male HFD fed offspring of either normotensive dams (120.3 ± 1.3 mm Hg) or hypertensive dams (128.7 ± 1.6 mm Hg), intact female offspring had a relatively smaller increase in MAP ($P < 0.05$), indicating sex differences in MHT-induced HTRS elicited by postweaning HFD (Table 3). Data are as follows (sex difference in intact females versus males: $F=86.46$ [5.000, 25.25]; $P < 0.0001$).

Ovariectomy augmented MAP in LFD fed offspring (normotensive-dam offspring versus hypertensive-dam offspring, 115.7 ± 1.4 versus 116.1 ± 0.7 mm Hg; $P < 0.05$) and HFD fed offspring of normotensive dam (127.1 ± 2.2 mm Hg; $P < 0.05$), but not in HFD fed offspring of hypertensive dam (122.5 ± 1.9 mm Hg; $P > 0.05$; Figure 5B and 5C) when compared with corresponding intact female offspring. The MHT-induced sensitization of BP in HFD-fed intact offspring was lost after ovariectomy when compared with HFD-fed ovariectomized offspring of normotensive dam, but still maintained higher than that in LFD offspring of both normotensive and hypertensive dam (intact and ovariectomized females: $F=28.85$ [7.000, 23.94]; $P < 0.0001$). Furthermore, the sex difference in the MHT-induced HTRS elicited by HFD was also lost after ovariectomy in female offspring (Table 3). Data are as follows (comparison of HFD fed ovariectomized females versus HFD males: $F=12.42$ [5.000, 22.63]; $P < 0.0001$).

In intact female offspring, HRs were comparable in all 4 groups of offspring, but significantly higher than that in male offspring. Ovariectomy significantly reduced HR in all groups of female offspring and eliminated sex differences in HRs regardless of whether the offspring were from either normotensive

Table 2. Metabolic Parameters in Intact and Ovariectomized Female Offspring of Normotensive and Hypertensive Dams

Parameter	Female offspring	Normotensive LFD	Normotensive HFD	Hypertensive LFD	Hypertensive HFD
Weaning body weight, g	Intact	48.3±2.2 (N=12)		47.0±2.3 (N=12)	
	Ovariectomized	47.0±3.4 (N=12)		48.8±2.4 (N=12)	
Body weight at week 12, g	Intact	221.4±6.4	268.1±8.1 ^{††}	249.7±5.2 [*]	277.4±4.4 ^{*†}
	Ovariectomized	302.0±7.9 [§]	337.4±9.8 ^{†§}	300.0±8.7 [§]	333.4±7.4 [§]
Body weight changes, g	Intact	173.1±6.4	219.7±8.1 ^{††}	202.5±5.1 [*]	229.8±4.4 ^{*†}
	Ovariectomized	251.2±7.4 [§]	292.7±8.6 ^{†§}	250.3±6.9 [§]	281.7±10.0 [§]
Food intake, g/d	Intact	11.3±0.2	8.9±0.2 [†]	12.2±0.2	9.2±0.1 [†]
	Ovariectomized	13.3±0.3 [§]	9.8±0.2 [†]	13.3±0.1 [§]	9.4±0.1 [†]
Energy intake, calories/d	Intact	43.4±0.7	46.4±1.1	46.9±0.9	48.4±0.4 [*]
	Ovariectomized	51.1±1.1 [*]	51.2±1.3 [*]	51.3±0.5 [*]	49.3±0.7 [*]
Feed efficiency, mg body weight/calorie	Intact	47.7±2.2	56.0±0.8 [*]	51.4±0.4 [*]	56.5±0.6 [*]
	Ovariectomized	58.7±0.8 [§]	67.3±0.8 ^{†§}	58.1±0.9 [§]	68.0±1.9 ^{†§}
Total fat mass, g	Intact	15.3±0.9	23.1±1.0 [*]	17.9±0.9	24.0±1.0 [*]
	Ovariectomized	20.9±2.0	34.5±2.4 ^{†§}	19.8±1.8	29.8±2.0 [†]
% Fat	Intact	6.9±0.3	8.8±0.3 ^{*†}	7.2±0.3	9.1±0.4 ^{*†}
	Ovariectomized	6.9±0.6	10.8±0.6 ^{†§}	6.6±0.4	8.8±0.4 [†]

$P < 0.05$ is significant. HFD indicates high-fat diet; and LFD, low-lard-fat diet.

^{*}Value vs intact normotensive LFD offspring.

[†]Value vs intact hypertensive LFD offspring.

^{††}Value vs both intact and ovariectomized LFD offspring.

[§]Value vs corresponding intact female offspring.

dams or hypertensive dams (Figure 5D, 5E, and 5F and Table 3). Data are as follows ($F[7, 34]=16.08$; $P < 0.0001$).

Changes in Autonomic Function After Diet Treatment in Female Intact and Ovariectomized Offspring

Ganglionic blockade (hexamethonium; 30 mg/kg; IP) did not result in a greater reduction in MAP in HFD-fed intact offspring when compared with LFD-fed intact offspring of either normotensive or hypertensive dams. However, ganglionic blockade induced significant reductions in MAP in all groups of ovariectomized offspring when compared with their corresponding intact offspring ($P < 0.05$). Furthermore, the reductions of MAP were greater in HFD-fed ovariectomized offspring than that in LFD-fed ovariectomized offspring ($P < 0.05$). These results suggest that ovariectomy removes an inhibitory constraint on SNS drive that leads to an increased sympathetic outflow from central nervous system in female offspring, and HFD feeding potentiates this process (Figure 6A). Data are as follows ($F [7, 34]=35.20$; $P < 0.0001$).

HR responses to either muscarinic receptor blockade ($F[7, 34]=0.8494$; $P=0.5510$) or β -adrenergic receptor antagonism ($F[7, 34]=0.5737$; $P=0.7721$) were similar in all groups ($P > 0.05$), suggesting that maternal hypertension, HFD, or ovariectomy had no effects

on cardiac sympathetic (Figure 6B) or vagal tone (Figure 6C).

Effect of Intracerebroventricular Injection of ANG II, TNF- α , or Leptin on BP in Diet-Treated Intact and Ovariectomized Offspring

In the intact offspring, both intracerebroventricular ANG II (Figure 7A and 7C) and TNF- α (Figure 8A and 8C) induced a greater pressor response in HFD-fed offspring of hypertensive dams when compared with the other 3 groups ($P < 0.05$), and there were no significant differences in pressor responses among these 3 groups. Ovariectomy significantly augmented the pressor response to intracerebroventricular ANG II (Figure 7B and 7C) or TNF- α (Figure 8B and 8C) only in HFD-fed offspring of normotensive dam, and pressor responses to intracerebroventricular ANG II or TNF- α remained higher in HFD-fed ovariectomized offspring of hypertensive dam when compared with LFD-fed intact offspring ($P < 0.05$). Data are as follows (ANG II: $F=12.56 [7.000, 18.51]$; $P < 0.0001$; TNF- α : $F=7.658 [7.000, 25.74]$; $P < 0.0001$).

There were no significant differences in pressor responses to intracerebroventricular leptin in all groups, suggesting that MHT, HFD feeding, or ovariectomy had no effects on the pressor response to intracerebroventricular leptin (Figure 9A through 9C). Data are as follows (leptin: $F[7, 32]=0.6506$; $P=0.7111$).

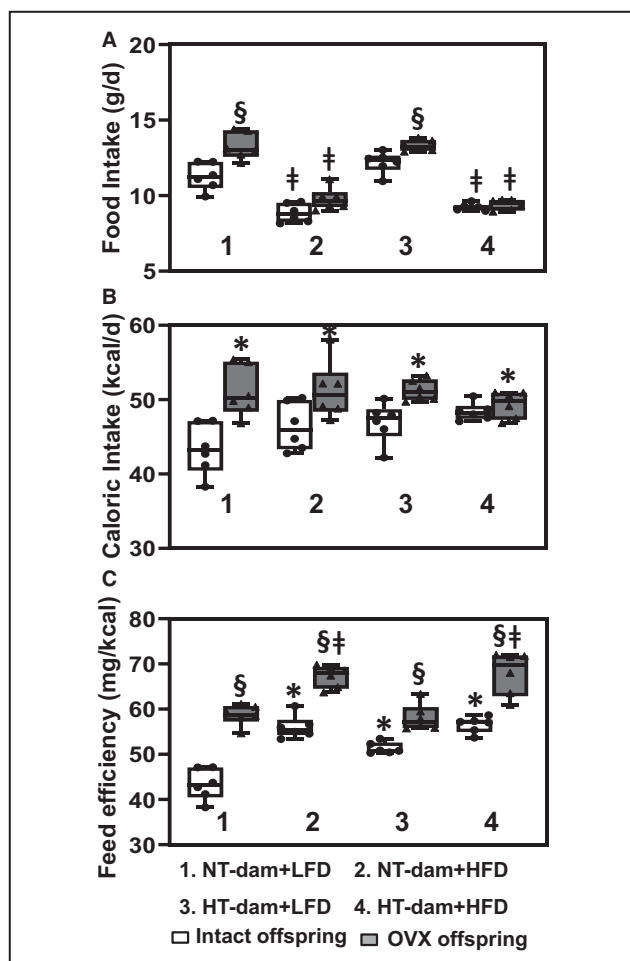


Figure 3. Food intake (A), caloric intake (B), and feed efficiency (C) in intact and ovariectomized (OVX) female offspring from normotensive (NT) dams and hypertensive (HT) dams after low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

Data are expressed as means±SEM, n=6/group; 2-way ANOVA was used for analysis followed by Tukey post hoc tests. * $P<0.05$ vs NT-LFD offspring; † $P<0.05$ vs intact or OVX LFD offspring; ‡ $P<0.05$ vs corresponding intact offspring.

Effect of HFD Feeding and Ovariectomy on Plasma Levels of ANG II, IL-6, and Leptin in Offspring

Plasma levels of ANG II were significantly increased only in HFD fed intact offspring of hypertensive dams when compared with LFD fed intact offspring ($P<0.05$). Ovariectomy elevated ANG II levels in HFD fed offspring of normotensive dams and remained high in HFD fed offspring of hypertensive dams when compared with LFD fed intact offspring of normotensive dams (Figure 10A). Data are as follows ($F=10.65$ [7.000, 38.77]; $P<0.0001$).

HFD feeding significantly elevated plasma levels of IL-6 only in intact offspring of hypertensive dams when compared with LFD fed offspring of hypertensive

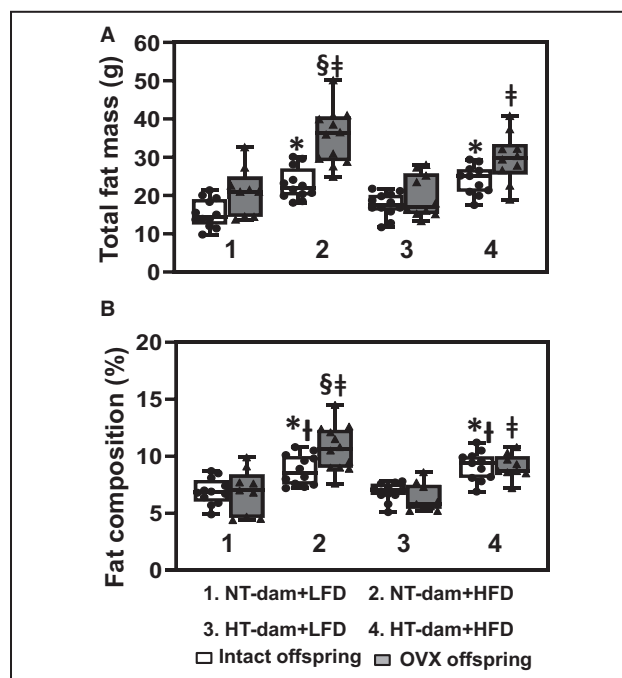


Figure 4. Fat mass (A) and fat composition (B) in intact and ovariectomized (OVX) female offspring from normotensive (NT) dams and hypertensive (HT) dams after low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

Data are expressed as means±SEM, n=10 to 12/group; 2-way ANOVA was used for analysis followed by Tukey post hoc tests. * $P<0.05$ vs NT-LFD offspring; † $P<0.05$ vs HT-LFD offspring; ‡ $P<0.05$ vs intact or OVX LFD offspring; § $P<0.05$ vs corresponding intact offspring.

dams. Ovariectomy significantly increased plasma levels of IL-6 in HFD fed offspring of normotensive dams and maintained high levels of IL-6 in HFD fed offspring of hypertensive dams (Figure 10B). Data are as follows ($F=4.125$ [7.000, 30.48]; $P=0.0027$).

HFD feeding significantly elevated plasma levels of leptin in intact offspring from either normotensive or hypertensive dams when compared with LFD feeding. However, ovariectomy increased the plasma levels of leptin in LFD fed offspring and deleted the differences in levels of leptin between LFD fed offspring and HFD fed offspring (Figure 10C). Data are as follows ($F=12.24$ [7.000, 24.38]; $P<0.0001$).

Effect of MHT, Postweaning HFD, and Ovariectomy on mRNA Expression of RAS Components, PICs, and Leptin in the PVN

RT-PCR analysis revealed that neither MHT nor HFD had effects on mRNA expression of ACE in intact offspring. However, ovariectomy resulted in significant increases in mRNA expression of ACE in all groups of offspring, and the increased expression of ACE was greater in HFD fed ovariectomized offspring of normotensive dams when compared with other groups

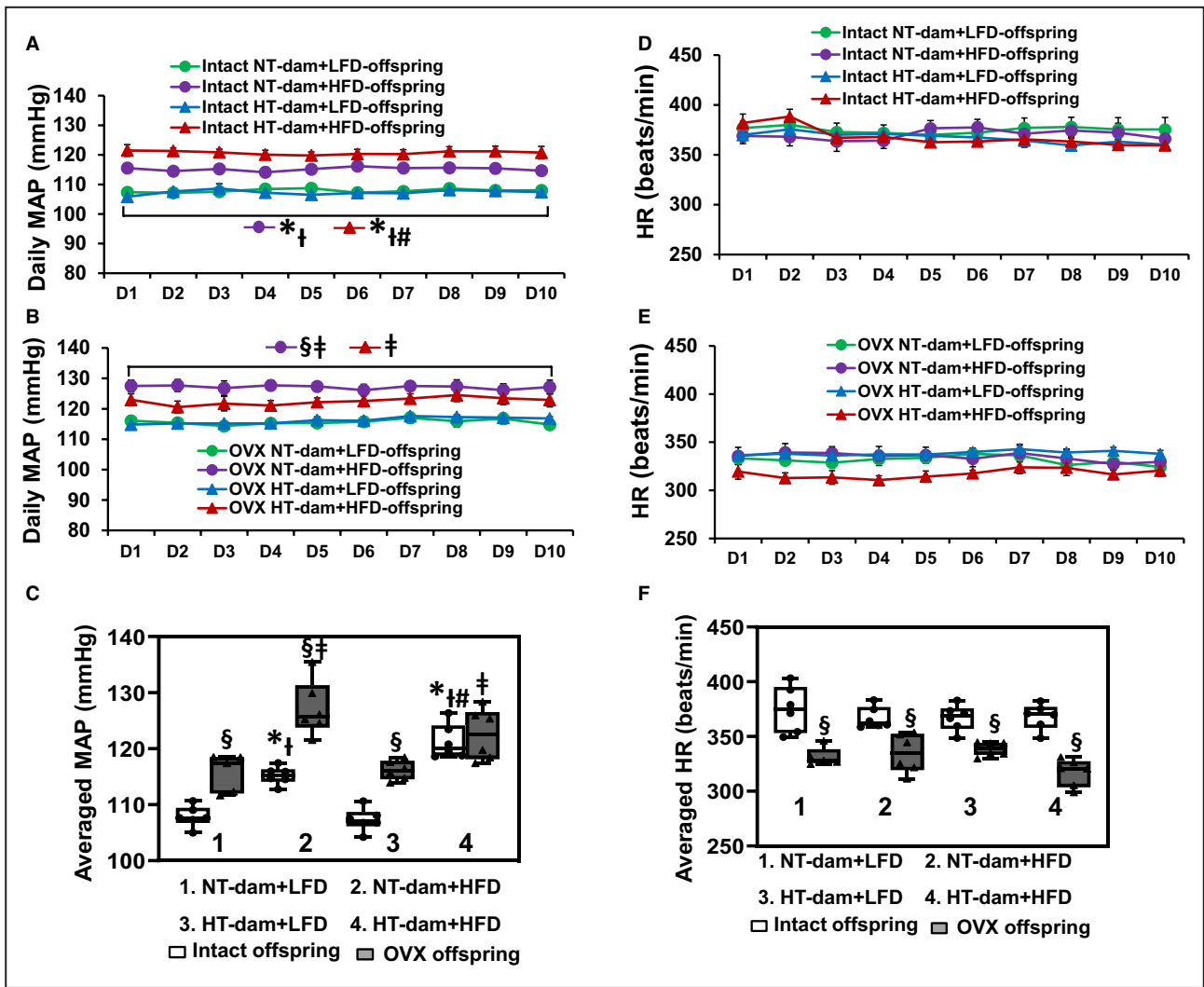


Figure 5. Effect of 12 weeks high-fat diet (HFD) feeding beginning from weaning on mean arterial pressure (MAP) and heart rate (HR) in intact (A–C) and ovariectomized (OVX; D–F) female offspring from normotensive (NT) dams and hypertensive (HT) dams.

Data are expressed as means±SEM, n=5 to 6/group; Brown-Forsythe and Welch ANOVA tests were used for MAP analysis, followed by Dunnett post hoc tests. Two-way ANOVA was used for HR analysis, followed by Tukey post hoc tests. **P*<0.05 vs NT-LFD offspring; †*P*<0.05 vs HT-LFD offspring; ‡*P*<0.05 vs NT-HFD offspring; §*P*<0.05 vs intact or OVX LFD offspring; #*P*<0.05 vs corresponding intact offspring.

of offspring ($F[7, 28]=15.23$; $P<0.0001$; Figure 11A). In contrast, maternal hypertension upregulated angiotensin II type 1 receptor expression in both LFD and HFD fed intact offspring, and ovariectomy eliminated this increased expression of angiotensin II type 1 receptor, which was comparable to that in ovariectomized offspring of normotensive dams ($F[7, 28]=14.16$; $P<0.0001$; Figure 11B). For leptin mRNA levels in the PVN, HFD feeding significantly elevated its expression in intact offspring from either normotensive or hypertensive dams when compared with LFD feeding. Ovariectomy eliminated the increased expression of leptin in HFD fed offspring and deleted the differences in leptin expression between LFD fed offspring and HFD fed

offspring ($F[7, 28]=29.71$; $P<0.0001$; Figure 11C). These data suggest that in intact offspring, MHT upregulated angiotensin II type 1 receptor expression in the PVN while ovariectomy upregulated ACE expression in all groups, especially in HFD fed offspring of normotensive dams, which may be involved in increased BP in these offspring.

Both MHT and HFD elicited a significant increase in mRNA expression of microglial marker CD11b (Figure 11D), TNF- α (Figure 11E), and IL-6 (Figure 11F) in the PVN of intact offspring. Moreover, the expression of TNF- α and IL-6 was greater in HFD fed offspring of hypertensive dams than HFD fed offspring of normotensive dam. Ovariectomy resulted in further increases

Table 3. MAP and HR in Male and Intact and Ovariectomized Female Offspring of Normotensive and Hypertensive Dams

Variable		Normotensive LFD	Normotensive HFD	Hypertensive LFD	Hypertensive HFD
Males		N=6	N=6	N=6	N=6
	MAP, mm Hg	112.7±1.0	120.3±1.3 ^{*†§}	112.6±0.9	128.7±1.6 ^{*†‡§}
	HR, beats/min	334.1±5.4 [§]	338.9±5.4 [§]	329.3±4.6 [§]	330.6±6.8 [§]
Intact female		N=6	N=6	N=6	N=6
	MAP, mm Hg	107.9±0.9	115.2±0.7 ^{*†}	107.3±0.8	121.3±1.3 ^{*†‡}
	HR, beats/min	374.9±8.6	366.9±4.0	367.0±4.8	367.9±4.8
Ovariectomized female		N=5	N=6	N=6	N=6
	MAP, mm Hg	115.7±1.4 [§]	127.1±2.2 ^{*†§}	116.1±0.7 [§]	122.5±1.9 ^{*†}
	HR, beats/min	331.3±3.5 [§]	334.7±7.9 [§]	338.6±2.6 [§]	317.2±5.1 [§]

The male MAP and HR data presented in this table were collected under exactly the same conditions as the female data. The male data were published previously³⁰ and are presented herein to facilitate comparison with results obtained in females. $P<0.05$ is significant. HFD indicates high-fat diet; HR, heart rate; LFD, low-lard-fat diet; and MAP, mean arterial pressure.

*Value vs intact normotensive LFD offspring.

†Value vs intact hypertensive LFD offspring.

‡Value vs intact normotensive HFD offspring.

§Value vs corresponding intact female offspring.

in CD11b expression in LFD fed offspring and HFD fed offspring of normotensive dam. The increased CD11b expression in HFD fed ovariectomized offspring of normotensive dams was greater than the other groups ($F[7, 28]=8.973$; $P<0.0001$; Figure 11D). However, ovariectomy eliminated the MHT and HFD-induced increase in TNF- α expression ($F[7, 28]=19.13$; $P<0.0001$; Figure 11E) but induced increased expression of IL-6 in ovariectomized offspring of normotensive dams and remained high IL-6 expression in ovariectomized offspring of hypertensive dams ($F[7, 28]=12.14$; $P<0.0001$; Figure 11F). These results indicate that in intact offspring, TNF- α and IL-6 exhibited higher mRNA expression in HFD fed offspring of hypertensive dams than HFD fed offspring of normotensive dams. In contrast, ovariectomy mainly elevated microglial activation and upregulated IL-6 expression in HFD fed offspring of normotensive dams. The altered cytokine expression may be related to higher BP in HFD fed intact offspring of hypertensive dams and in HFD fed ovariectomized offspring of normotensive dams.

For mRNA expression of putative antihypertensive components, both MHT and HFD upregulated mRNA expression of IL-10 in the PVN of intact female offspring. Ovariectomy significantly reduced increased expression of IL-10 in offspring of hypertensive dams but had no effects on offspring of normotensive dams ($F[7, 28]=11.83$; $P<0.0001$; Figure 11G). Similarly, both MHT and HFD upregulated mRNA levels of Mas-R in the PVN of intact offspring, and ovariectomy eliminated this increased expression ($F[7, 28]=26.81$; $P<0.0001$; Figure 11H). In contrast, the mRNA expression of AT2-R was upregulated only in HFD fed intact offspring of normotensive dams. Ovariectomy significantly reduced AT2-R expression in all groups of offspring ($F[7, 28]=14.19$; $P<0.0001$; Figure 11I). The results suggest

that in intact offspring, either MHT or HFD upregulated the expression of these antihypertensive components, especially AT2-R only in HFD fed offspring of normotensive dams. However, ovariectomy downregulated these increased expressions, suggesting estrogen plays an important role in maintaining expression of the antihypertensive components and MHT impairs an estrogen-mediated AT2-R protective effect.

DISCUSSION

The major findings of the present study are as follows: (1) In postweaning HFD fed intact female offspring, MHT induced HTRS and enhanced pressor responses to centrally administered ANG II and TNF- α , but not to leptin. (2) The HFD-elicited increase in BP in MHT sensitized female offspring was less than that seen in male offspring, but this sexual dimorphism was eliminated by ovariectomy. (3) Ovariectomy significantly enhanced the HFD-elicited increase in BP and the pressor responses to central ANG II or TNF- α in HFD-fed offspring of normotensive dam. In contrast, the sensitized BP and pressor response in HFD-fed intact offspring of hypertensive dams were not potentiated further after ovariectomy, but these parameters still remained high so that there were no differences between the HFD-fed ovariectomized offspring of normotensive and hypertensive dams. (4) The central nervous system originating cardiac sympathetic drive and vagal tone were not different between HFD-fed versus LFD-fed offspring. Ovariectomy induced increased centrally driven sympathetic outflow, and HFD feeding potentiated this response. (5) MHT and HFD upregulated mRNA expression of both prohypertensive and antihypertensive components in the PVN in intact offspring. Ovariectomy further enhanced several prohypertensive components and inflammatory

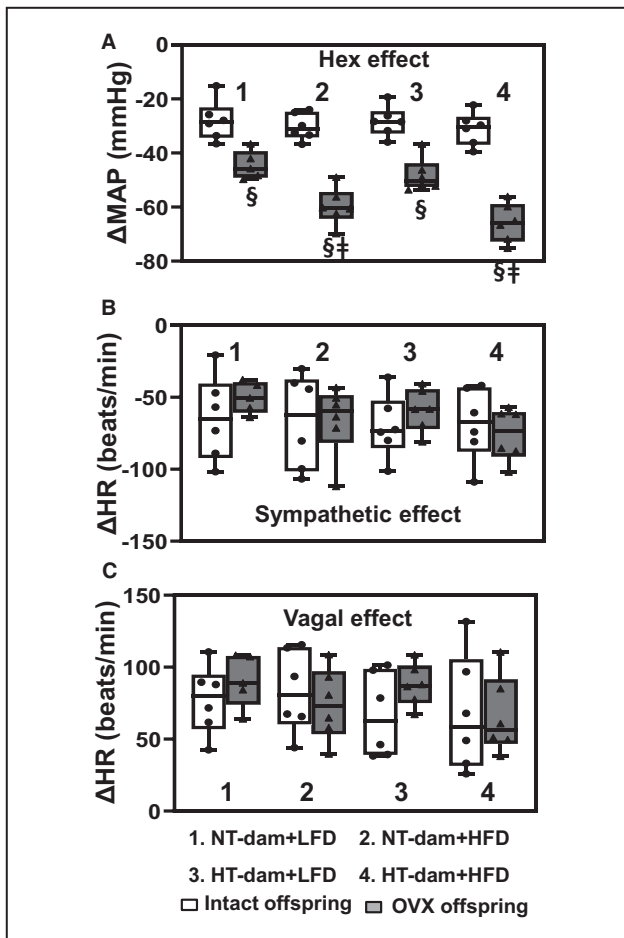


Figure 6. The changes in autonomic function in intact and ovariectomized (OVX) female offspring from normotensive (NT) dams or hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding. The autonomic parameters, including centrally driven sympathetic tone (A), cardiac sympathetic activity (B), and cardiac vagal activity (C), were obtained by intraperitoneal injection of hexamethonium (Hex), atenolol, and atropine, respectively. Data are expressed as means±SEM, n=5 to 6/group; 2-way ANOVA was used for analysis, followed by Tukey post hoc tests. [†]P<0.05 vs intact or OVX LFD offspring; [§]P<0.05 vs corresponding intact offspring.

mediators, including ACE, IL-6, and the microglial marker CD11b in HFD fed offspring of normotensive dams, but downregulated antihypertensive components in all offspring. Collectively, the results indicate that estrogen is a key sex hormone accounting for the sex differences in HTRS and that it plays a critical role in antagonizing increases in body weight gain and fat mass and HFD prohypertensive effects. MHT compromises a normal protective action of estrogen against HTRS elicited by HFD in female offspring. The loss of the protective action of estrogen alters the balance of central antihypertensive and prohypertensive components, resulting in augmented brain reactivity to centrally applied pressor agents.

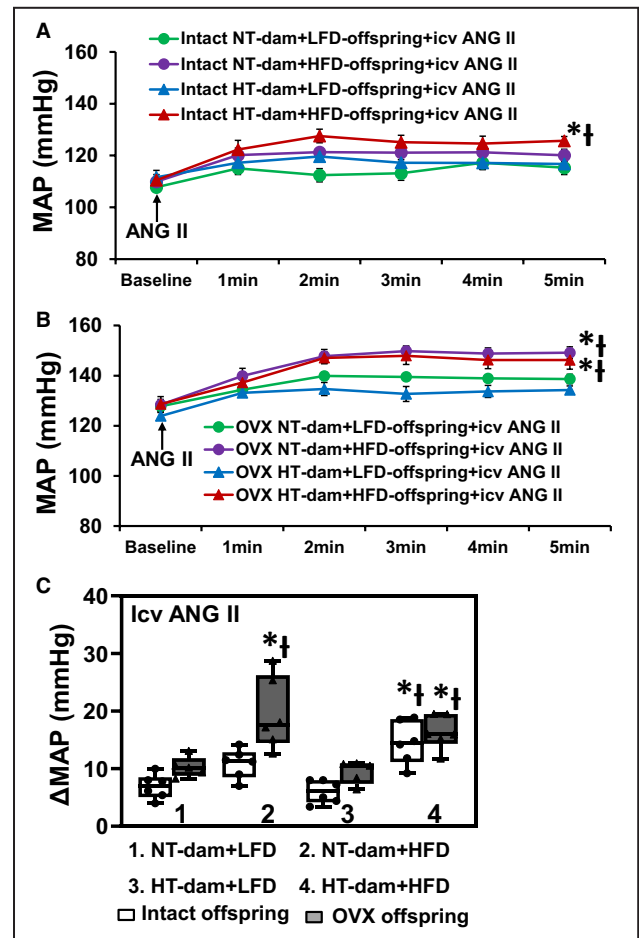


Figure 7. The pressor effects of intracerebroventricular (icv) injection of angiotensin (ANG) II in intact (A and C) and ovariectomized (OVX; B and C) female offspring from normotensive (NT) dams or hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding. A and B, Group data, showing blood pressure responses to icv injection of the pressor agent. C, Bar graph, showing changes in mean arterial pressure (MAP). Data are expressed as means±SEM, n=5 to 6/group; Brown-Forsythe and Welch ANOVA tests were used for analysis of changes in MAP, followed by Dunnett post hoc tests. ^{*}P<0.05 vs NT-LFD offspring; [†]P<0.05 vs HT-LFD offspring.

Substantial evidence from many different models of prenatal insult indicates that the developmental programming of hypertension in offspring is sex specific and characterized by earlier onset, increased severity, or enhanced pressor responses in male offspring in response to a second challenge (“second hit”), such as HFD.^{1,2,10,18,19,21,23} In previous studies, we used a maternal ANG II–induced hypertension during pregnancy model to determine whether sensitization of the hypertensive response could be produced in the next generation, and found that there was sexual dimorphism in the hypertensive response elicited by administration of a slow-pressor dose of ANG II.^{10,31} Recently, several

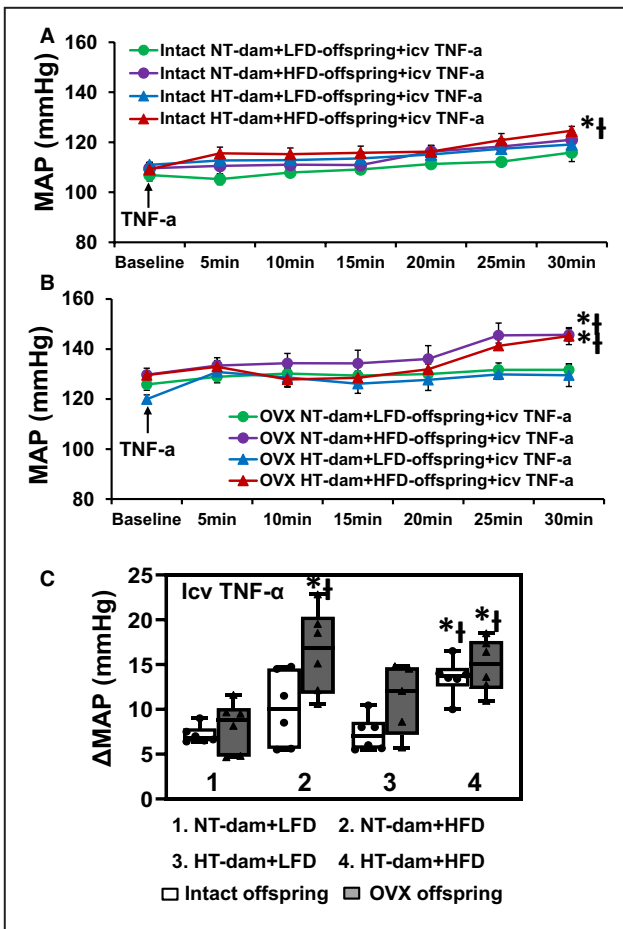


Figure 8. The pressor effects of intracerebroventricular (icv) injection of tumor necrosis factor (TNF)-α in intact (A and C) and ovariectomized (OVX; B and C) female offspring from normotensive (NT) dams or hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

A and B, Group data, showing blood pressure responses to icv injection of the pressor agent. C, Bar graph, showing changes in mean arterial pressure (MAP). Data are expressed as means±SEM, n=5 to 6/group; Brown-Forsythe and Welch ANOVA tests were used for analysis of changes in MAP, followed by Dunnett post hoc tests. *P<0.05 vs NT-LFD offspring; †P<0.05 vs HT-LFD offspring.

studies tested if adverse effects of an abnormal intra-uterine environment on BP are magnified by changing the postnatal environment with an obesogenic diet as a second challenge.^{18,19,21,23,32} Although all of these studies found sex differences in prenatal insult-induced increase in BP, one study showed that BP was increased in HFD/high-sugar diet groups, but that the increases in BP were similar for HFD/high-sugar fed male or female growth-restricted offspring versus same-sex control counterparts.¹⁹ Another study demonstrated that postnatal HFD increased vulnerability of prenatal dexamethasone-induced hypertension in male but not in female adult offspring.¹⁸ However, Sellayah et al reported that maternal protein restriction leads to

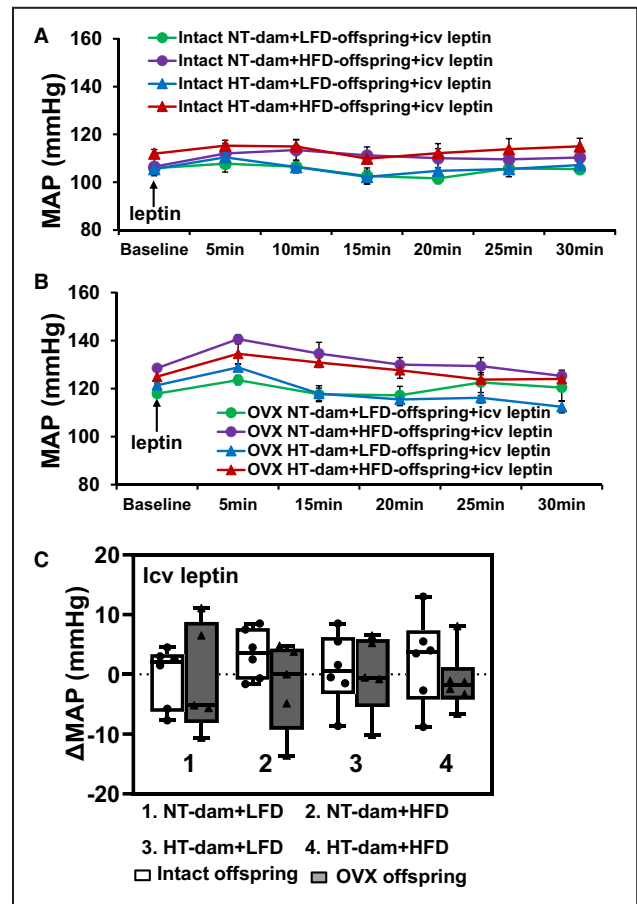


Figure 9. The pressor effects of intracerebroventricular (icv) injection of leptin in intact (A and C) and ovariectomized (OVX; B and C) female offspring from normotensive (NT) dams or hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

A and B, Group data, showing blood pressure responses to icv injection of the pressor agent. C, Bar graph, showing changes in mean arterial pressure (MAP). Data are expressed as means±SEM, n=5 to 6/group; 2-way ANOVA was used for analysis, followed by Tukey post hoc tests. P>0.05.

elevation in BP, which is exacerbated by a postweaning HFD in both male and female offspring.²³ Using female offspring exposed *in utero* to MHT induced by endothelial NO synthase knockout in mice, Longo and colleagues demonstrated a cardiometabolic-like syndrome phenotype, including higher BP after postnatal HFD feeding.³² Consistent with these 2 latter studies, we found that maternal ANG II-induced hypertension resulted in a HTRS to postweaning HFD in female offspring with a sex-specific manner, suggesting that the female offspring are relatively protected from the synergistic effects of MHT and postnatal HFD compared with their male littermates. Discrepancies in sex differences in postnatal HFD exacerbation of prenatal insult-induced hypertension among these studies, including ours, may depend on the use of different prenatal insult models.

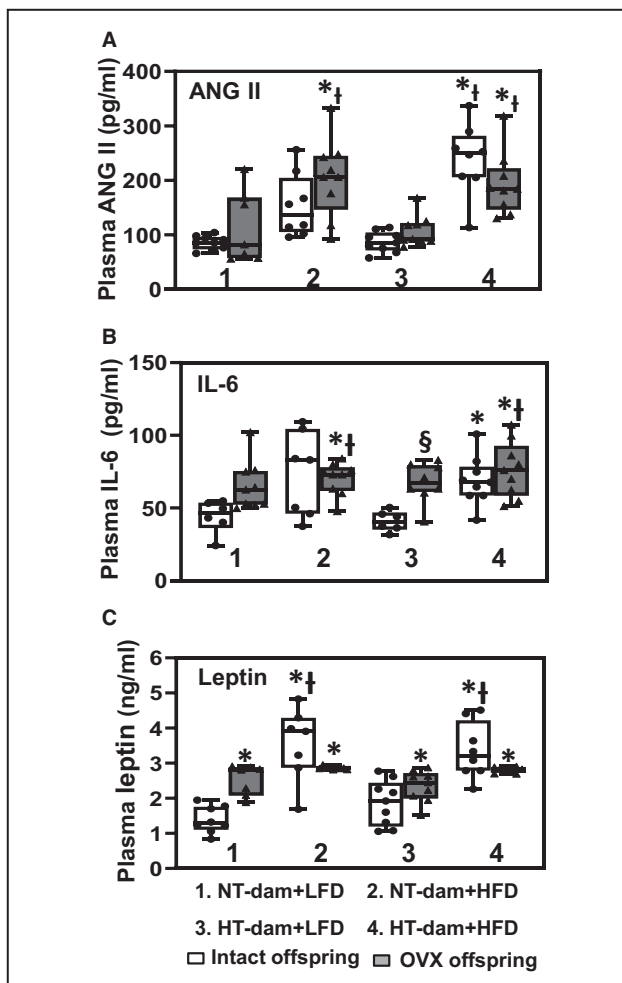


Figure 10. Comparisons of plasma angiotensin (ANG) II (A), interleukin 6 (IL-6) (B), and leptin (C) in intact and ovariectomized (OVX) female offspring from normotensive (NT) dams or hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

Data are expressed as means \pm SEM, n=6 to 9/per group; Brown-Forsythe and Welch ANOVA tests were used for analysis of plasma levels of agents, followed by Dunnett post hoc tests. * P <0.05 vs NT-LFD offspring; † P <0.05 vs HT-LFD offspring; § P <0.05 vs corresponding intact offspring.

Estrogen and testosterone, either alone or together, are likely to be responsible for the sex differences in the development of hypertension in offspring of different prenatal insult models.^{7-9,11,12} Our previous study showed that unlike male offspring, females from hypertensive dams did not respond to ANG II with a greater hypertensive response than the female offspring of normotensive dams. Ovariectomy, but not castration, altered the hypertensive response to ANG II in offspring from hypertensive dams, showing a greater BP increase in the ovariectomized offspring of hypertensive dams than the ovariectomized offspring of normotensive dams.¹⁰ However, in the present study, we found that in HFD fed intact

female offspring, BP was greater in offspring of hypertensive dams than that in offspring of normotensive dams. Ovariectomy augmented an increase in BP in HFD fed offspring of normotensive dams, but ovariectomy did not alter the sensitized BP increase elicited by HFD in offspring of hypertensive dams. Ovariectomy eliminated the BP differences between HFD fed female offspring of normotensive and hypertensive dams and between HFD fed male and ovariectomized female offspring. These data suggest that female sex hormones are the major factor responsible for the sexual dimorphism of BP in the prenatal hypertension–postnatal HFD model and play a protective effect against HFD-induced increase in BP in offspring from normotensive dams. MHT itself disrupts a normal protective action of estrogen in postweaning HFD fed intact offspring, resulting in a sensitized BP response to postnatal HFD.

Obesity/HFD is a major risk factor for hypertension, with an increase in SNS activation serving the link between activation of the RAS and inflammation and the increase in BP.¹³⁻¹⁵ These multiple factors also contribute to the effects of aversive developmental programming of hypertension.^{12,15,21,33} However, it is necessary to consider the origins of increased SNS drive that contribute to the increased BP in offspring exposed to prenatal insult and postnatal HFD. Evidence indicates that estrogen protects against various forms of hypertension through inhibiting a glial neuroinflammatory response and central nervous system inflammation,³⁴ upregulating the antihypertensive pathway and inhibiting the prohypertensive pathway both peripherally and centrally.^{15,26} In the present study, we found that in intact female offspring of normotensive dams, HFD induced increases in plasma levels of ANG II and IL-6 and in mRNA expression of PICs in the PVN, but that the pressor responses to central ANG II or TNF- α were not elevated. In contrast, in HFD fed intact female offspring of hypertensive dams, the expression of RAS and PIC components was enhanced and the pressor responses to central ANG II or TNF- α were augmented, which was likely responsible for the MHT-induced HTRS to HFD in female offspring. These data suggest that estrogen has a protective effect against HFD-induced elevation of brain reactivity mediated by upregulation of the RAS and inflammation, and that MHT impaired the protective action of estrogen, resulting in increased brain reactivity. This point is further supported by our ovariectomy data showing that estrogen deficiency enhanced pressor responses in HFD fed offspring of normotensive dams and maintained a higher pressor response in HFD fed offspring of hypertensive dams, which was accompanied by enhanced centrally driven sympathetic activity and expression of RAS and PIC components in the brain. Accordingly, the increase in BP was enhanced in HFD offspring of

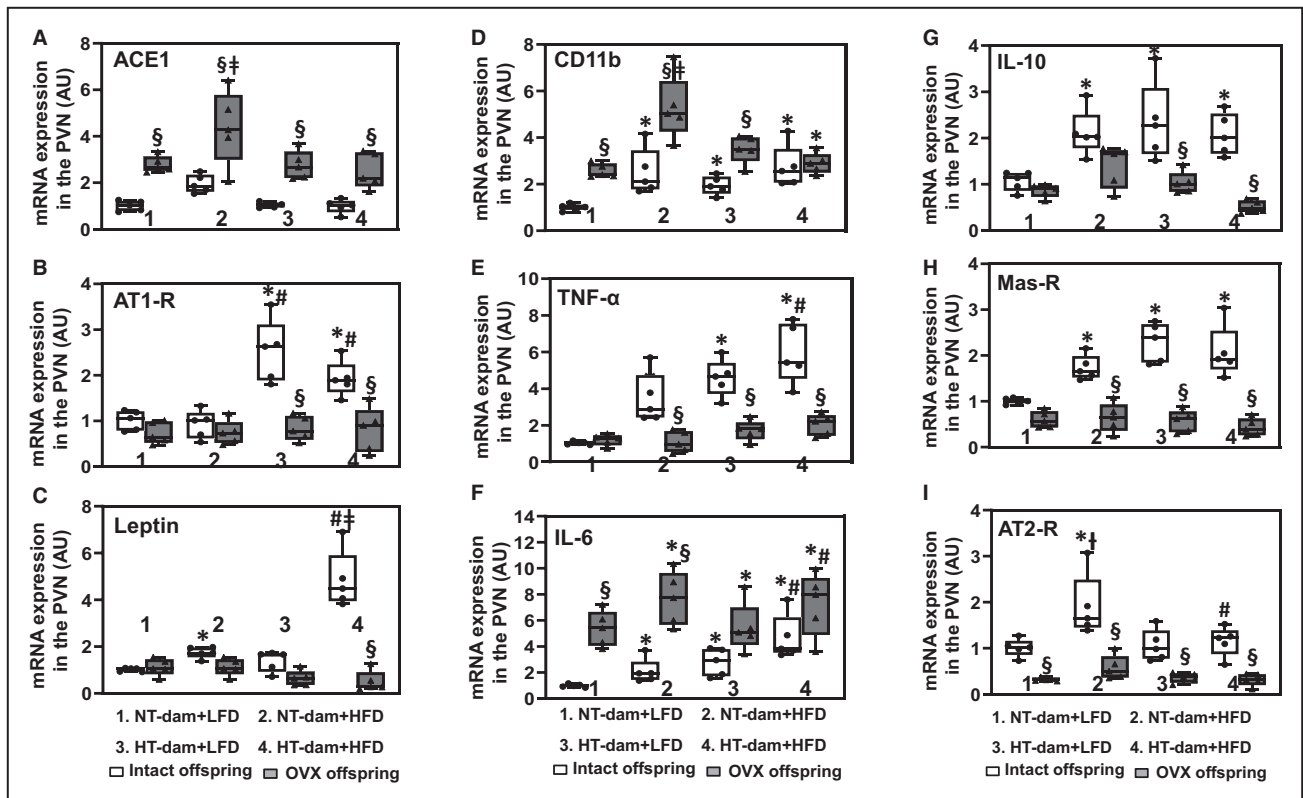


Figure 11. Comparison of the mRNA expression of renin-angiotensin system components (A, B, H and I), proinflammatory and anti-inflammatory cytokines (D, E, F and G), and leptin (C) in the paraventricular nucleus (PVN) of intact and ovariectomized (OVX) female offspring from both normotensive (NT) and hypertensive (HT) dams after 12 weeks low-lard-fat diet (LFD) or high-fat diet (HFD) feeding.

Data are expressed as means±SEM, n=5/group; 2-way ANOVA was used for analysis, followed by Tukey post hoc tests. *P<0.05 vs NT-LFD offspring; †P<0.05 vs HT-LFD offspring; ‡P<0.05 vs NT-HFD offspring; §P<0.05 vs corresponding intact offspring; #P<0.05 vs HT-HFD offspring.

normotensive dams and the BP differences were eliminated between HFD fed offspring of normotensive and hypertensive dams.

Besides the inhibitory effect of estrogen on the prohypertensive pathway, estrogen activation of an antihypertensive pathway is another mechanism protecting females against HFD-associated hypertension.³⁵ Recent studies have demonstrated that in response to an HFD, the RAS antihypertensive pathway involving ACE2, angiotensin-(1–7), and Mas-R was activated and prevented an increase in BP in intact female mice. Blockade or knockout of the Mas-R abolished protection of female mice from obesity-induced hypertension. Estrogen plays a major role in mediating increased expression of this pathway component in adipose tissue.^{36–38} Another RAS antihypertensive pathway involving ANG II and the AT2-R can also potentially mediate estrogen protection against ANG II- or aldosterone-induced hypertension in female animals.^{39–41} Estrogen replacement, in aged, reproductively senescent female mice, blunted the pressor response in an ANG II-induced model of hypertension. This effect was associated with upregulation of renal

AT2-R, which was abrogated by AT2-R antagonism.³⁹ In the present study, MHT and HFD upregulated mRNA expression of the Mas-R and IL-10, an antihypertensive cytokine, in the PVN of intact female offspring. Especially, the expression of AT2-R was upregulated in HFD fed offspring of normotensive dams but not of hypertensive dams, suggesting that the effect of estrogen on the AT2-R was impaired by MHT. Furthermore, ovariectomy significantly reduced the expression of all these antihypertensive components in the PVN, suggesting that estrogen is required for activation of antihypertensive pathway. The present study provides evidence of a central mechanism underlying estrogen protection against MHT-induced HTRS to postnatal HFD through regulation of brain antihypertensive and prohypertensive pathways that leads to inhibition of brain reactivity to pressor agents.

The complex mechanisms involving systemic or central interactions between female sex hormones with leptin have been investigated in obese females. Alexander and colleagues demonstrated that age induces an increase in visceral fat and circulating leptin associated with a significant increase in BP in female intrauterine growth-restricted

12-month-old offspring. In this study, the renal nerves are implicated as a mediating mechanism, suggesting that there are important interactions among the factors of aging, estrogen deficiency, and leptin producing increased sympathetic activity and BP.⁴² However, data from Shi et al suggest that the differential effects of leptin between the sexes are not attributable to different sites or pathways of action, but rather a positive interaction between leptin and estrogen at the cellular level.^{15,25} Huby et al. reported that leptin is required for obesity-associated hypertension and that aldosterone mediates the increased BP in young female mice.⁴³ In the current study, we studied relatively young female offspring fed with HFD (15–16 weeks of age versus 12 months in other studies) and did not find elevated sympathetic activity in intact female offspring or an enhanced pressor response to central leptin in intact and ovariectomized offspring. This suggests that leptin may not be involved in postweaning HFD-induced change in BP in female offspring, but did in the male offspring,³⁰ even though there were changes in plasma levels and brain mRNA expression of leptin in HFD fed female offspring before and after ovariectomy. On the basis of aforementioned studies and current results, it is likely that the actions of leptin in obesity-related hypertension in females exposed to prenatal insults may depend on the animal model studied, age, species, and whether the females are in prereproductive or postreproductive senescence.^{15,16} Further investigations on these issues are warranted in the future.

It is well established that estrogen plays an important role in preventing body weight gain in females.^{44,45} The reduction of endogenous estrogen by ovariectomy in female animals leads to increased body weight gain and fat mass, and these obese phenotypes can be prevented by estrogen replacement.^{44,45} Various prenatal insults lead to metabolic dysfunction, and postnatal HFD exacerbates it.^{19,23,32} In the present study, we found that both maternal hypertension and HFD feeding similarly increased body weight and feed efficiency in intact female offspring. However, MHT had no effects on fat accumulation, showing no differences in fat mass and composition between HFD fed offspring of normotensive and hypertensive dams. These results indicate that MHT was associated with changes in body weight gain, but not in adiposity in offspring, which is consistent with the metabolic results from other prenatal insult plus postnatal HFD models.^{19,23} Furthermore, although the impaired effect of estrogen on fat mass and composition was not evident in HFD fed intact offspring of hypertensive dams, ovariectomy resulted in a significant increase in the fat mass and composition in HFD offspring from normotensive dams but not from hypertensive dams when compared with corresponding HFD intact offspring. These data suggest that the loss of estrogen uncovered its regulatory effect on fat mass in HFD fed offspring of normotensive dams, which is consistent with the BP changes in the offspring after

ovariectomy. The estrogenic effects on energy balance are believed to be primarily mediated by brain estrogen receptor (ER)- α .⁴⁶ ER α is abundantly expressed in multiple brain regions that are implicated in the regulation of body weight balance. These include the ventrolateral portion of the ventromedial hypothalamus, the arcuate nucleus, the medial preoptic area, and the nucleus of the solitary tract.⁴⁷ The PVN is a key nucleus that integrates RAS and PIC signaling to activate the SNS and elevate BP. However, the PVN predominately expresses ER β .^{24,26,29} Therefore, it may be that estrogen protects against MHT-induced HTRS and regulates body weight and fat mass through separate pathways, possibly involving different receptor subtypes (ER α versus ER β) and different brain regions (the PVN versus other nuclei involved in energy metabolism). Our results indicating that there were differences in increases of BP but no differences in body weight and fat mass between HFD fed intact female of normotensive and hypertensive dams support this point.

In summary, the offspring of mothers with gestational hypertension exhibit subclinical disturbances that can be unmasked after exposure to a second stressor (“a second hit”), such as eating an HFD. The present study demonstrated that postnatal consumption of an HFD in female as well as male offspring of mothers with gestational hypertension produces a sensitized hypertensive response. The hypertensive response was attenuated somewhat in females compared with males, and this sexual dimorphism was attributable to the protective effect of estrogen. MHT impairs the protective effect of estrogen against postweaning HFD-associated increase in BP through shifting balance of prohypertensive pathway and antihypertensive pathway in favor of an enhanced pressor mode in the brain so that brain reactivity driving the SNS is increased to pressor agents. Our findings provide insight into the central cardiovascular protective mechanisms present in females involving estrogen that acts to decrease risk of developmental programming of hypertension induced by prenatal and postnatal environmental insults.

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Disclosures

None.

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