ORIGINAL RESEARCH

Female Mice Exposed to Postnatal Neglect Display Angiotensin II–Dependent Obesity-Induced Hypertension

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Background—We have previously reported that female mice exposed to maternal separation and early weaning (MSEW), a model of early life stress, show exacerbated diet-induced obesity associated with hypertension. The goal of this study was to test whether MSEW promotes angiotensin II–dependent hypertension via activation of the renin-angiotensin system in adipose tissue.

Methods and Results—MSEW was achieved by daily separations from the dam and weaning at postnatal day 17, while normally reared controls were weaned at postnatal day 21. Female controls and MSEW weanlings were placed on a low-fat diet (LF, 10% kcal from fat) or high-fat diet (HF, 60% kcal from fat) for 20 weeks. MSEW did not change mean arterial pressure in LF–fed mice but increased it in HF–fed mice compared with controls ($P<0.05$). In MSEW mice fed a HF, angiotensin II concentration in plasma and adipose tissue was elevated compared with controls ($P<0.05$). In addition, angiotensinogen concentration was increased solely in adipose tissue from MSEW mice ($P<0.05$), while angiotensin-converting enzyme protein expression and activity were similar between groups. Chronic enalapril treatment (2.5 mg/kg per day, drinking water, 7 days) reduced mean arterial pressure in both groups of mice fed a HF ($P<0.05$) and abolished the differences due to MSEW. Acute angiotensin II–induced increases in mean arterial pressure (10 μg/kg SC) were attenuated in untreated MSEW HF–fed mice compared to controls ($P<0.05$); however, this response was similar between groups in enalapril-treated mice.

Conclusions—The upregulation of angiotensinogen and angiotensin II in adipose tissue could be an important mechanism by which female MSEW mice fed a HF develop hypertension. **(*J Am Heart Assoc*. 2019;8:e012309. DOI: 10.1161/JAHA.119.012309.)**

Key Words: adipose tissue • hypertension • maternal separation • obesity • renin-angiotensin system

The obesity epidemic affects 1 of every 3 adults in the United States,[1,2] and when analyzed by sex, the statistics show that women across all ages display higher percentages of obesity than men.[3–5] Obesity and health disparities are two interrelated health concerns that begin in early life.[6–9] National studies have demonstrated disparities in health status in minority youth, including significantly higher mortality rates of metabolic and cardiovascular disease.[10–12]

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Among the well-documented psychosocial and environmental factors influencing lifestyle and well-being, early life stress has been established as an independent risk factor for increased chronic diseases.[13–15] Recent studies have highlighted the negative effects of adverse childhood experiences on the development of obesity and elevated systolic blood pressure during adulthood.[14,16] Moreover, there is a growing body of literature showing that early life stress experiences are associated with a higher prevalence of obesity in women than in men.[17–21]

Middle-aged women with an early life history of physical and sexual abuse are more likely to show higher body mass index and frequency of binge eating when compared with nonabused women.[22] Accordingly, it has been shown that sexual, physical, and emotional abuse independently predicts waist circumference in adult women.[23,24] In a cross-sectional study performed in women of childbearing age, prepregnancy obesity was associated with a self-reported history of emotional or physical abuse.[25] Additionally, body mass index trajectories indicate a faster rate of weight gain in women who experienced trauma or posttraumatic stress disorder symptoms as adults, relative to nonexposed women.[26] These

Clinical Perspective

What Is New?

- The development of cardiovascular disease is strongly influenced by lifestyle and environmental factors that determine the impact of traditionally defined risk factors, and this study provides important new understanding about the underlying mechanisms linking early life stress with increases in fat mass and high blood pressure.
- Using a mouse model of neglect, maternal separation and early weaning, we demonstrated that female mice develop angiotensin II–dependent, obesity-induced hypertension.
- Fat expansion and elevated blood pressure are associated with an activation of adipose tissue and systemic renin-angiotensin system, suggesting that maternal separation and early weaning primes adipose tissue to be a source of angiotensin II in female mice fed a high-fat diet.

What Are the Clinical Implications?

- In light of growing evidence showing that women exposed to early life stress are at higher risk for obesity and metabolic dysfunction, this study further supports a sex-specific modulation of the renin-angiotensin system within adipose tissue as a potential therapeutic target to lower cardiovascular disease risk.

findings reflect the recognized prevalence of stress, depression, obesity, and heart disease comorbidities observed more often in women compared with men.

The activation of the renin-angiotensin system (RAS) is one of the major mechanisms implicated in the development and progression of obesity-induced hypertension by increasing angiotensin II circulating levels.[27] Various tissues in the body express most of the RAS components, including adipose tissue, where production of angiotensin II from its precursor, angiotensinogen, contributes to obesity-induced hypertension in male mice.[28] Deletion of angiotensinogen in adipose tissue, or angiotensin type 1 receptor in kidney and brain, have successfully prevented the onset of hypertension in models of experimental obesity.[29–33] Moreover, obesity-induced hypertension has been linked to increases in adipose-derived angiotensin II in male mice.[30,31] In females, several studies have reported that chronic HF does not elevate blood pressure despite being associated with increases in fat mass.[30,32,34,35] Yet it has been demonstrated that female mice are able to develop hypertension in genetic models of obesity.[36]

Postnatal maternal separation and early weaning (MSEW) is an experimental mouse model used to establish the effects of neglect in early life. We have previously shown that MSEW increases blood pressure in response to a chronic high-fat diet (HF) in male and female mice.[37] However, only female MSEW mice display exacerbated fat expansion and metabolic derangements. Hence, this study was designed to test the hypothesis that MSEW predispose female mice to develop obesity-induced hypertension via an increased capacity of adipose tissue to produce angiotensin II. Thus, we determined the RAS status in plasma and tissues (hepatic, renal, and adipose) of control and MSEW mice fed a low-fat diet (LF) or HF. In addition, we investigated the effects of angiotensin-converting enzyme (ACE) inhibitor treatment on blood pressure and the acute blood pressure sensitivity to angiotensin II. Finally, we studied the potential contribution of the autonomic nervous system on the mechanism by which MSEW impairs the protection against obesity-induced hypertension in female mice.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal Model

All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and were approved and monitored by the Institutional Animal Care and Use Committee at the University of Kentucky. Mice were given ad libitum access to food and water while housed in a pathogen-free environment, with constant temperature and humidity, and a 14:10 hour light:-dark cycle. MSEW protocol was performed in C57BL/6J mice (The Jackson Laboratory) as described previously.[37] Briefly, pups were separated from the dams by transferring the pups to a clean cage inside an incubator (30±1° C, humidity 60%) for 4 hours from postnatal day 2 to day 5 and for 8 hours from postnatal day 6 to day 16 of life with early weaning at postnatal day 17. Normally reared, nonhandled litters that remained with the dams served as control groups and were weaned at postnatal day 21. Only litters with 6 to 8 pups were used in the experiments. Each litter was derived from a different breeding pair to avoid litter effects. Entire litters were maintained until weaning; female littermates were randomized for telemetry and blood pressure/tissue collection at weaning and male littermates were used for other projects.

Experimental Design

Dams were fed a regular chow diet (Teklad 8604, Madison, WI). At weaning, female offspring were fed a LF or HF (10% or 60% kcal from fat, D12450J and D12492, respectively; Research Diets, Inc, NJ) for 20 weeks. After 16 weeks on the diet, a subset of mice (n=6/group) were implanted with radiotelemeters (PA-C10, Data Sciences, Inc, St. Paul, MN) for blood pressure and heart rate measurements as described

previously.[37] After 15 days of recovery, baseline blood pressure was recorded for 5 consecutive days. Then, autonomic function was determined in conscious animals as well as the acute effect of increasing doses of angiotensin II before and after treatment with enalapril (2.5 mg/kg per day, drinking water, 7 days), an ACE inhibitor, on blood pressure. The other subset of littermates fed a LF or HF (n=6–8/group) were used as time controls. At the end of the experiments, mice were anesthetized with ketamine/xylazine (150/20 mg per kg IP) for exsanguination by cardiac puncture and tissue harvesting.

Plasma Renin and Angiotensinogen Concentrations

Plasma renin concentration (PRC) was quantified by incubating mouse plasma (8 μL) with exogenous angiotensinogen (25 nmol/L) prepared from nephrectomized rats (30 min) in phosphate buffer containing EDTA (0.05 mol/L) and enalapril (10 μmol/L), followed by quantification of angiotensin I by radioimmunoassay as previously described.[31,33] Plasma and tissue angiotensinogen were determined using an ELISA kit following the manufacturer's protocol (Immuno-Biological Laboratories America, Minneapolis, MN).

Plasma and Tissue Angiotensin II by Radioimmunoassay

Perigonadal white adipose tissue (gWAT, ≈30 mg) and renal tissue (kidney cortex, ≈50 mg) were placed in chilled microcentrifuge tubes containing 400 μL of radioimmunoassay buffer, without BSA, in the presence of cOmplete Protease Inhibitor Cocktail (Roche, Basel, Switzerland) and 2 steel Geno/Grinder balls (SPEX SamplePrep, Metuchen, NJ). Fat and kidney extracts were prepared by adding 0.8 mL of buffer to the samples and using the Geno/Grinder as the homogenization method. After preparing the extracts, 100 μL of extract was used per tube in the radioimmunoassay. Tissue samples were homogenized using the GenoGrinder for 1 minute ×1350 rpm. After the steel balls were removed, samples were centrifuged at 16000*g* ×10 minutes, 4°C. Supernatants were transferred to clean tubes. A 100 μL-aliquot of each extract was used per assay tube. The radioimmunoassay was performed using I-125 radio-iodinated angiotensin II tracer (prepared for Dr Lisa Cassis by Robert Speth, PhD, Nova Southeastern University, Ft. Lauderdale, FL), as reported by Hunter and Greenwood,[38] and angiotensin II antibody (#T-4005, Peninsula Laboratories International, Inc, San Carlos, CA) was diluted to a concentration of 0.03 μg/100 μL per tube. A concentrated solution of BSA was added to reduce autolysis and for a final concentration of 2 mg/mL. To obtain plasma angiotensin II concentraions, % B/Bo of samples (as percent of the amount of label [I125-AngII] bound to antibody divided by the amount of label binding in the absence of unlabelled antigen) was used to interpolate concentrations from a generated standard curve. Results are reported as picograms of angiotensin II per milligram of protein in the tissue extract used in the assay.

Angiotensin (1-7)

Concentrations of angiotensin (1-7) were measured using a commercial enzyme immunoassay, manufacturer's protocol IV (S-1330; Peninsula Laboratories International, Inc, San Carlos, CA). The cross-reactivity for this enzyme immunoassay is 100% for angiotensin I/II (1-7) and 0% for angiotensin I/II (1-5), I (1-9), I, II, III, and A.

Fat Tissue Explants Protocol

Under anesthesia, mice were perfused (heparinized NaCl, 0.9%) and adipose tissue was collected. Then, adipose tissue explants (gWAT, 40 mg) were incubated (1 hr at 37°C) in DMEM (200 μL) containing free fatty acid-BSA (1%). Angiotensinogen content in incubation media (1:100 dilution) and fat explants (40 mg, 1:5 dilution) were measured by ELISA, following the manufacturer's guidelines (Immuno-Biological Laboratories America).

ACE Protein Expression and Activity Measurement

ACE activity in gWAT was measured as previously described.[39] Briefly, fat samples were homogenized in buffer containing 20 mmol/L HEPES with 0.5% Triton X-100 (pH 7.3), and centrifuged at 20 000 g for 20 minutes at 4°C. The supernatant was collected and stored at −80°C for ACE activity assay. The protein concentrations of samples were determined by the Pierce BCA protein assay kit (ThermoFisher, Rockford, IL). ACE activity was measured using a fluorescence ACE assay, as previously described.[40] For this, 20 μg of protein extract was diluted to 100 μL in assay buffer (50 mmol/L HEPES pH 8, 200 mmol/L NaCl, 10 μM Zn acetate), and then 100 μL of the fluorogenic peptide substrate Mca-R-P-P-G-F-S-A-F-K(Dnp)-OH (R&D Systems, Netherlands) was added into each well at a concentration of 10 μmol/L in assay buffer with or without the ACE inhibitor lisinopril. The degradation of the fluorogenic peptide (fluorescence) was measured over time in a spectrophotometer (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany) at 320 nm excitation and 405 nm emissions. Only the hydrolytic activity inhibited by lisinopril was considered for calculations. ACE expression in gWAT was assessed by western blot. Briefly, protein homogenates were denatured, resolved,

transferred into polyvinylidene fluoride membranes, and then probed with a goat polyclonal antibody against ACE (Santa Cruz Biotechnology, Santa Cruz, CA). GAPDH was used as the protein loading control.

Angiotensin II Acute Response in Conscious Mice

A 1-hour baseline was recorded before the experiments. The acute subcutaneous administration of angiotensin II (0, 1, 10, and 50 μg/kg in sterile saline) was performed, allowing blood pressure to recover between doses. Fifteen minutes after each injection, a 5-minute average of systolic blood pressure (SBP) was reported as delta pressor response from baseline.

Autonomic Function in Conscious Mice

A 1-hour baseline was recorded before the experiments. After a baseline period, mice received an acute injection of mecamylamine (5 mg/kg, IP) or propranolol (5 mg/kg, IP) to study the effects of MSEW on sympathetic tone. In addition, an acute response to atropine (1 mg/kg, IP) was performed to test the effect of MSEW on the parasympathetic function. To determine the effects on blood pressure and heart rate (HR), 30 minutes after each injection, a 5-minute average was reported as delta pressor response from baseline.

Statistical Analysis

All data are presented as mean±SEM. Two-way ANOVA followed by Bonferroni post hoc test was used to assess the differences between control and MSEW mice in different dietary conditions. One-way ANOVA followed by Tukey's multiple comparisons test was used to analyze the differences in delta blood pressure from LF-fed mice and between untreated and enalapril-treated HF-fed mice. One-way repeated measures ANOVA followed by Tukey's was used to analyze the effects of angiotensin II dose. Three-way ANOVA was performed to assess the differences in response to angiotensin II (10 mg/kg) in untreated and enalapril-treated control and MSEW fed LF and HF. Analyses were performed using GraphPad Software version 7.00 (La Jolla, CA). Because of the small sample sizes per group, normality of outcomes is assumed. Statistical significance was determined by $P<0.05$.

Results

MSEW Increases Circulating Angiotensin II Concentrations in Female Mice Fed a HF

In accordance with our previous reports, body weight and fat mass were not different between control and MSEW LF-fed mice. However, HF significantly increased body weight and fat mass in female MSEW mice compared with controls (Table 1). Plasma angiotensinogen, renin concentration, and angiotensin II were similar between control and MSEW LF-fed mice. In mice fed a HF, plasma angiotensinogen levels were similarly increased in both groups (Figure 1A). MSEW mice, but not controls, displayed a reduction in PRC (Figure 1B). In addition, high-fat feeding increased circulating angiotensin II concentration in both groups; however, this increase was significantly higher in female MSEW mice compared with controls (Figure 1C). Plasma angiotensin (1-7) concentration was not different between control and MSEW mice fed a LF (0.26±0.04 versus 0.19±0.03 ng/mL, respectively), while HF increased angiotensin (1-7) levels similarly in both groups (0.43±0.09 versus 0.46±0.08 ng/mL, respectively).

MSEW Increases Angiotensin II Content in Adipose Tissue

Female MSEW HF-fed mice displayed a robust increase in gWAT angiotensin II concentration (Figure 2A), but no changes in renal cortex angiotensin II content were observed among the groups (Figure S1). To determine the source of angiotensin II, we measured the capacity of freshly isolated gWAT to generate angiotensinogen. Our results showed that angiotensinogen concentration in media explants were similar between control and MSEW mice, whereas a HF reduced its concentration similarly in both groups (Figure 2B). Interestingly,

Table 1. Body Composition in 20-Week-Old Control and MSEW Mice Fed a low-fat diet or HF

	LF		HF				
	Control	MSEW	Control	MSEW	*P* Inter	*P* Diet	*P* MSEW
Body weight, g	23.9±0.3	25.1±0.8	41.9±1.2*	45.9±0.8*,†	0.007	<0.0001	0.065
Fat mass (% BW)	13.8±0.9	17.7±1.6	33.7±1.5*	45.8±0.4*,†	0.015	<0.0001	<0.0001
Lean mass (% BW)	78.1±1.4	74.9±1.3	57.5±1.2*	44.0±0.9 *,†	0.008	<0.0001	<0.0001

Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. BW indicates body weight, HF, high-fat diet; LF, low-fat diet; MSEW, maternal separation and early weaning.
*$P<0.05$ vs. LF; †$P<0.05$ vs. control. n=8 per group.

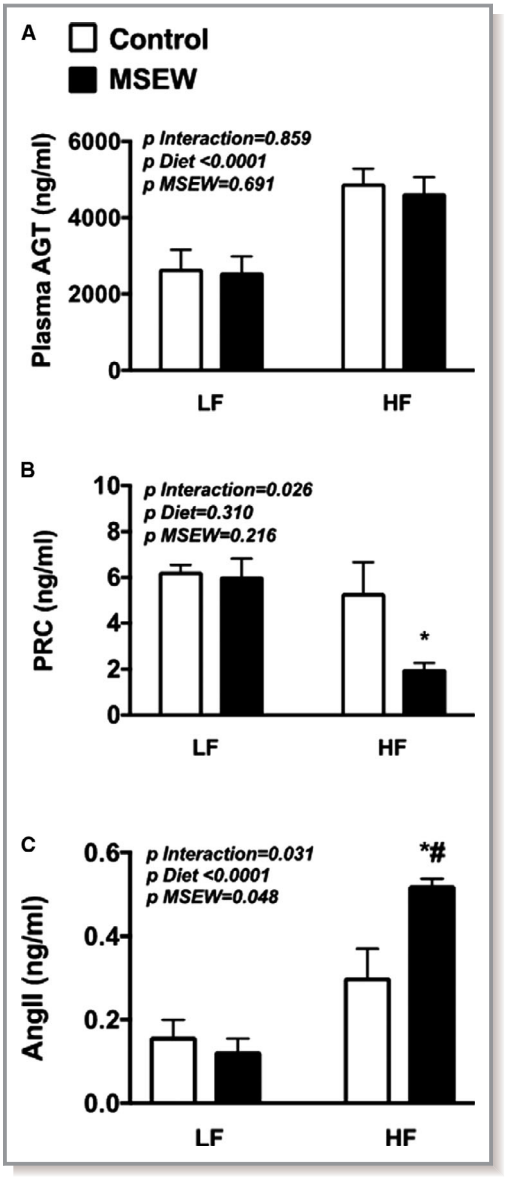

Figure 1. Effect of maternal separation and early weaning (MSEW) in plasma RAS components. **A**, Plasma angiotensinogen (ng/mL), (**B**) plasma renin concentration (PRC, ng/mL), and (**C**) angiotensin II concentration (ng/mL) in female control (white bars) and MSEW (black bars) mice fed a low-fat diet (LF) or a high-fat diet (HF). Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. **P*<0.05 vs. control, #*P*<0.05 vs. LF; n=8 per group in HF-fed mice.

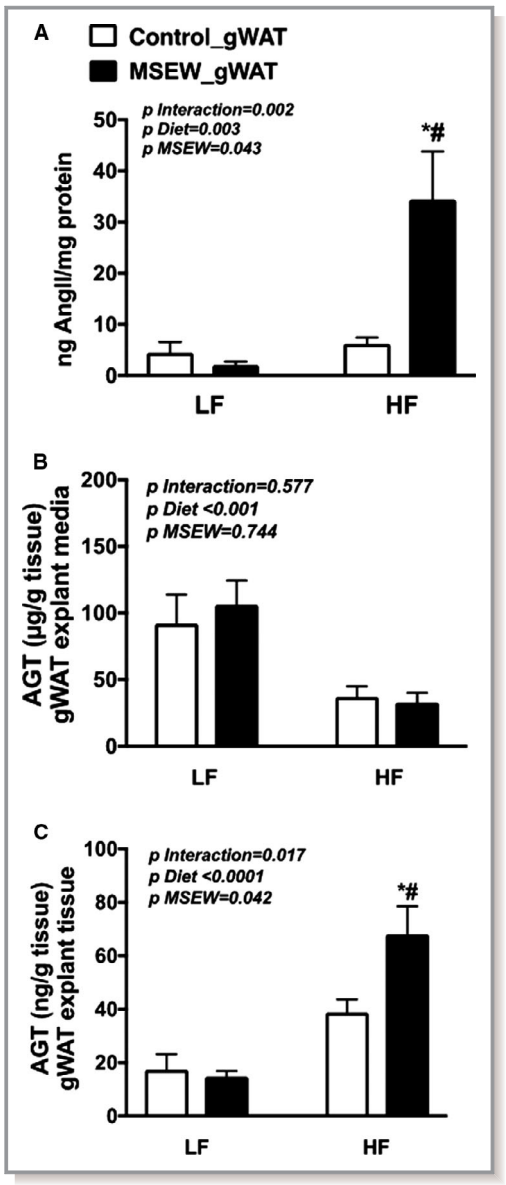

Figure 2. RAS components in gonadal white adipose tissue (gWAT). **A**, Angiotensin II concentration (angiotensin II, ng angiotensin II/mg protein), (**B**) angiotensinogen levels in fat explant media (µg/g); and (**C**) angiotensinogen levels in gWAT explant tissue (ng/g) in female control (white bars) and maternal separation and early weaning (MSEW) (black bars) mice fed a low-fat diet (LF) or a high-fat diet (HF). Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. **P*<0.05 vs. control, #*P*<0.05 vs. LF; (**A**) n=8 per group; (**B**) n=8 per group media explant; (**C**) n=6 per group tissue explant.

angiotensinogen concentration was significantly increased in tissue explants from MSEW (Figure 2C). In the liver and the kidney cortex, angiotensinogen content was similar between control and MSEW mice (Figure S2). No significant differences in ACE expression (Figure 3A, Figure S3) or activity (Figure 3B) were found attributable to diet or separation in female mice fed a HF.

MSEW Exacerbates AngII-Dependent Obesity-Induced Hypertension

Female control and MSEW mice fed a LF show similar mean arterial pressure (MAP), SBP, diastolic blood pressure, and HR,

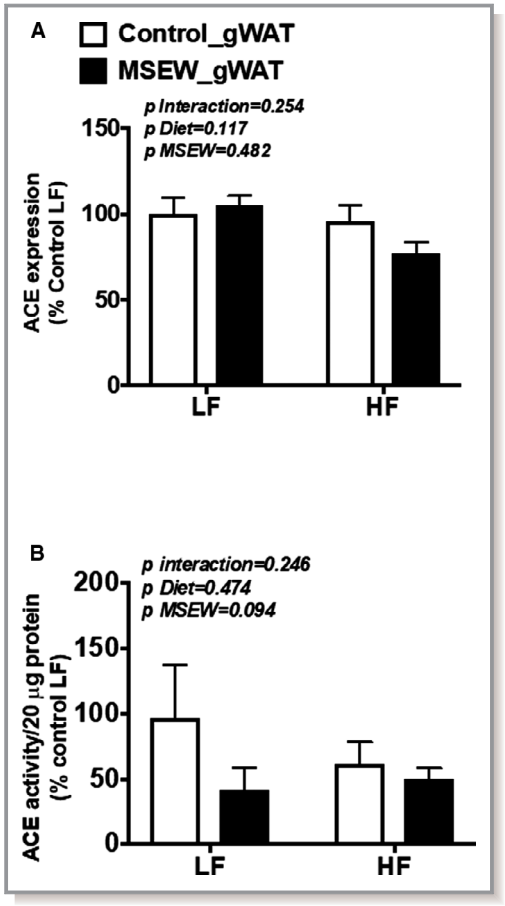

Figure 3. Effect of maternal separation and early weaning (MSEW) in angiotensin-converting enzyme (ACE). **A**, expression (% control LF) and (**B**) activity (20 µg protein) in perigonadal white adipose tissue (gWAT) of female control (white bars) and MSEW (black bars) mice fed a low-fat diet (LF) and high-fat diet (HF); n=3 control LF, 8=control HF, 4=MSEW LF, 8=MSEW HF.

while HF increased MAP and SBP in MSEW mice compared with controls (Table 2). In addition, HF increased HR in both control and MSEW mice (Table 2). We also determined the effect of HF as a delta pressor response from LF for each group of untreated mice. HF did not change MAP, SBP, and diastolic blood pressure in control mice (Figure 4A through 4C). Conversely, HF-induced increases in MAP and SBP were significantly greater in MSEW mice, whereas diastolic blood pressure was not influenced by the diet (Figure 4A through 4C). In mice fed a HF, chronic enalapril administration significantly decreased MAP in both control and MSEW (101±3 and 100±3 mm Hg, respectively; $P<0.05$ versus untreated), abolishing the differences between the groups. Enalapril-treated control and MSEW mice also showed reduced SBP (116±3 and 115±7 mm Hg, respectively; $P<0.05$ versus untreated) and diastolic blood pressure (85±3 versus 84±3 mm Hg, respectively; $P<0.05$ versus untreated). There was no effect of enalapril on HR in control and MSEW females (592±11 versus 615±12 bpm, respectively) compared with the untreated mice. Additionally, a similar blood pressure change was observed in response to enalapril treatment in mice fed a HF (Figure 4D through 4F). As anticipated, enalapril administration dramatically reduced angiotensin II concentration in plasma in both HF-fed control and MSEW females (0.16±0.03 and 0.13±0.04 ng/mL, respectively; $P<0.05$ compared with values in Figure 1C).

MSEW Does Not Affect Angiotensin II–Induced Blood Pressure Sensitivity in Mice Fed a HF

In untreated control mice fed a HF, there was a dose-response effect to acute angiotensin II on SBP after 15 minutes (Figure S4). MSEW mice showed an attenuated SBP response, which was significantly reduced at 10 µg/kg angiotensin II dose. Therefore, we compared the acute blood pressure response to 10 µg/kg angiotensin II dose in LF- and HF-fed mice.

We performed a 3-way ANOVA; however, the interaction between *diet* × *MSEW* × *treatment* failed to reach statistical significance ($P=0.896$). This could be attributable to the small sample size per group and, in consequence, the low power of the analysis. Therefore, we use 2-way ANOVA by treatment to better understand the effect of the 2-way interactions.

Table 2. Blood Pressure and Heart Rate in 20-Week-Old Control and MSEW Mice Fed a LF or HF

	LF		HF				
	Control	MSEW	Control	MSEW	*P* Inter	*P* Diet	*P* MSEW
MAP, mm Hg	108±2	106±3	112±2	117±2*,†	0.004	0.0001	0.030
SBP, mm Hg	127±1	126±2	129±1	136±3*,†	0.025	0.011	0.048
DBP, mm Hg	92±2	89±2	95±2	93±3	0.773	0.168	0.408
HR, bpm	574±12	558±13	611±7	616±8	0.252	0.0001	0.568

Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. BW indicates body weight; DBP, diastolic blood pressure; HF, high-fat diet; HR, heart rate; LF, low-fat diet; MAP, mean arterial pressure; MSEW, maternal separation and early weaning; SBP, systolic blood pressure.
*$P<0.05$ vs. LF, †$P<0.05$ vs. control HF. n=6 per group.

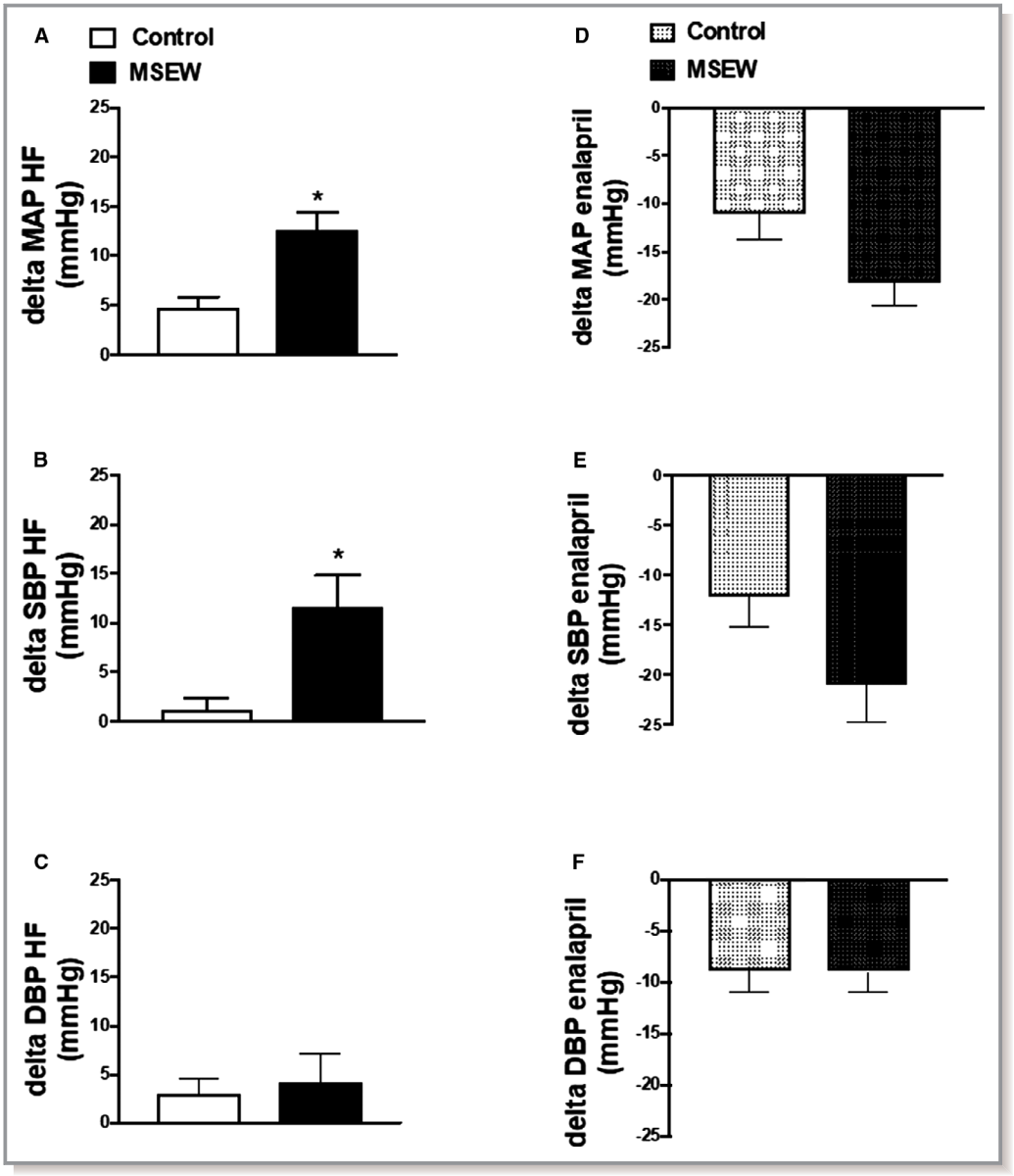

Figure 4. Effect of diet or angiotensin-converting enzyme (ACE) inhibitor on blood pressure. Delta blood pressure changes from LF to HF in female control (white solid bars) and maternal separation and early weaning (MSEW) (black solid bars): (**A**) Mean arterial pressure (MAP, mm Hg); (**B**) Systolic blood pressure (SBP, mm Hg); (**C**) Diastolic blood pressure (DBP, mm Hg). Delta blood pressure changes from untreated to enalapril-treated HF-fed female control (white dotted bars) and MSEW (black dotted bars): (**D**) MAP; (**E**) SBP; (**F**) DBP. Data were analyzed by 1-way ANOVA followed by Tukey's post hoc test and reported as mean±SEM.*$P<0.05$ vs. control; n=6 per group.

Untreated MSEW mice fed a LF showed reduced SBP compared with controls; however, this response was further decreased in MSEW mice fed a HF (Figure 5A). Following the inhibition of the endogenous conversion of angiotensin I to angiotensin II with enalapril, control and MSEW mice showed a similar response to acute angiotensin II–induced increases in SBP (Figure 5B), although these responses were ≈15 mm Hg lower in HF-fed mice compared with LF-fed mice. These data suggest that the inhibition of endogenous angiotensin II synthesis by ACE abolished the effect of MSEW on the acute blood pressure response.

MSEW Does Not Influence Autonomic Function in Mice Fed a HF

To evaluate autonomic function, we induced acute blood pressure changes in conscious, untreated mice fed a HF using compounds that block sympathetic and parasympathetic

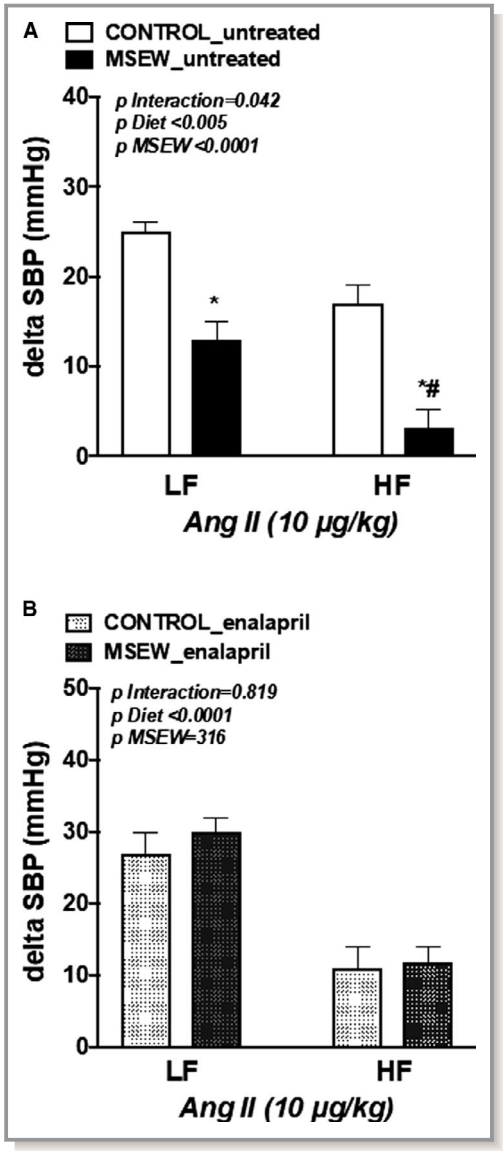

Figure 5. Effect of HF on acute angiotensin II–induced pressor response (10 ng/kg, SC) in female control (white bars) and maternal separation and early weaning (MSEW) (black bars) mice. **A**, Delta systolic blood pressure changes (delta SBP, mm Hg) in untreated (solid bars) and (**B**) enalapril-treated (dotted bars). Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. **P*<0.05 vs. control, #*P*<0.05 vs. LF, n=6 per group.

responses (Table 3). No significant differences between control and MSEW were found in MAP and HR response induced by ganglion blockade with mecamylamine. Likewise, the propranolol-induced reduction in HR was similar in control and MSEW HF-fed mice. In addition, atropine-induced blockade of parasympathetic tone did not show a significant effect on MAP and HR in either group. These data suggest that increased obesity-induced hypertension in MSEW mice could be independent of alterations in sympathetic and parasympathetic function.

Discussion

We found that increased adiposity and blood pressure in female MSEW mice fed a HF are associated with an activation of the adipose and systemic RAS. Consistent with these findings, chronic ACE inhibition abolished MAP elevations in MSEW mice, supporting a primary role for increased angiotensin II in the development of hypertension in these mice. In addition, increased angiotensin II levels in plasma from MSEW mice is most likely attributable to the greater capacity of the adipose tissue to generate angiotensin II via increases in its precursor, angiotensinogen. Thus, our study shows that, in female mice, exposure to MSEW during postnatal life may prime the adipose tissue RAS to generate angiotensin II in response to a HF.

Early life stress, defined as adverse childhood experiences during the first decade of life, has been established as an independent risk factor for increased body mass index and blood pressure.[13,16,41,42] Importantly, several studies have shown that the association between obesity (visceral or retroperitoneal fat) and the development of hypertension and cardiovascular disease is established early in life.[4,43] MSEW is a mouse model that mimics the negative effects of early life stress on behavioral, neuroendocrine, and cardiovascular responses in humans.[37,44,45] Our laboratory has previously shown that MSEW exacerbates obesity-induced hypertension in both male and female mice. In addition, our studies in rodents demonstrated that exposure to maternal separation exerts a sex-specific effect on fat deposition.[37,46,47] While maternal separation has no effect on fat mass expansion and body weight gain in response to a HF in male rats and mice,[47,48] females display elevated visceral fat mass, upregulation of hepatic adipogenic gene expression, and insulin resistance. We also have shown that increased adiposity in female rats exposed to maternal separation can be blunted by postnatal treatment with a corticosterone synthase inhibitor.[37,47] It is important to mention that these increases are not related to differences in food intake.[37] Taken together, these data suggest that adipose tissue from female MSEW mice is primed to expand in response to chronic HF and to produce vasoactive factors. The association between obesity and increased visceral or retroperitoneal fat is a well-established independent risk factor for the development of hypertension and cardiovascular disease.[4,43] Typically, adipose tissue responds to environmental stressors (or stress hormones) by secreting adipokines, RAS components, and cytokines.[49–53] One potential explanation for this sex-specific susceptibility to store fat in the visceral depot in response to chronic stress could be linked to the fact that females are predisposed to gain weight with possible evolutionary origins related to reproductive efficiency.[54,55] Therefore, postnatal MSEW-induced increases in glucocorticoids could exacerbate this response.

Table 3. Autonomic Nervous System Response in Female Control and MSEW Mice Fed a HF

	Mecamylamine		Propranolol		Atropine	
	Control	MSEW	Control	MSEW	Control	MSEW
Baseline MAP, mm Hg	100±2	104±3	96±3	100±4	104±3	108±4
Response MAP, mm Hg	76±3	79±4	120±2	123±5	112±4	118±4
Delta MAP, mm Hg	−23±4	−31±3	24±4	23±3	8±1	7±2
Baseline HR, bpm	580±12	592±5	558±7	575±8	535±17	525±7
Response HR, bpm	536±17	556±7	515±9	527±12	586±22	554±7
Delta HR, bpm	−43±8	−36±6	−42±11	−58±6	34±6	29±6

Sympathetic response in female control and MSEW mice fed a HF in response to mecamylamine (5 mg/kg) and propranolol (5 mg/kg). Parasympathetic tone was evaluated in response to atropine (1 mg/kg). Data were analyzed by 2-way ANOVA followed by Bonferroni post hoc test and reported as mean±SEM. n=6 per group. BW indicates body weight; HF, high-fat diet; HR, heart rate; LF, low-fat diet; MAP, mean arterial pressure; MSEW, maternal separation and early weaning.

We found that female MSEW mice display increased circulating angiotensin II concentration, whereas PRC is reduced compared with controls. In line with previous work,[31,33] a HF reduced PRC levels in female mice when compared with LF-fed mice, suggesting a negative feedback loop of renin in response to elevated angiotensin II. Therefore, our data suggest that higher angiotensin II levels in MSEW mice may exert a stronger inhibition on PRC. We also investigated the activation of RAS in adipose tissue as a potential source of angiotensin II. We found that female MSEW mice fed a HF display increases in angiotensin II concentrations compared with controls. We also found that gWAT from MSEW mice display greater amounts of angiotensinogen, with no changes in ACE protein expression or activity. These data support the notion that MSEW exacerbates angiotensin II–induced hypertension by influencing the production of the angiotensin II precursor. Previous studies demonstrated that overexpression of angiotensinogen in adipose tissue increased SBP in male mice,[56] while the deletion of angiotensinogen in adipose tissue reduced blood pressure in C57BL/6 mice.[30,57] As a result, angiotensinogen deficiency in adipose tissue of male mice fed a HF prevented obesity-induced elevations in plasma angiotensin II concentrations and SBP.[30] In our study, we determined that HF might stimulate angiotensinogen production specifically in the adipose tissue of female MSEW mice. Numerous studies have shown that glucocorticoids are a strong positive regulator of the angiotensinogen gene. In addition, angiotensinogen promoter activity has been shown to be influenced by changes in DNA methylation in response to, for instance, excess of circulating aldosterone.[58,59] Therefore, exposure to MSEW could prime the adipose tissue to produce angiotensinogen in response to a chronic HF feeding via epigenetic mechanisms. Other potential mechanisms contributing with the development of metabolic disease and related high blood pressure could be hormones and adipokines targeted by early life stress. Accordingly, we have previously reported that female MSEW mice display moderate increase in plasma leptin and aldosterone levels when fed a HF.[37] These results support further the findings by Huby et al,[36] showing that leptin is a positive regulator for aldosterone production in genetic models of leptin sensitization and obesity where female mice develop hypertension. Future studies will be focused on identifying adipose tissue–derived factors and epigenetic modifications that may play a role in specifically activating the RAS components in visceral adipose tissue.

Sex differences in tissue-specific RAS status are consistently reported in experimental models of obesity.[30,32,60] In accordance with our current findings, Gupte et al have shown that female C57BL/6J mice fed a HF are protected against the development of obesity-induced hypertension via the stimulation of the protective arm of the RAS (eg, ACE2, angiotensin [1-7], Mas receptor).[30] In contrast, HF-fed male mice showed obesity-induced hypertension, associated with elevated systemic angiotensin II levels, that is abolished in mice with adipocyte-specific angiotensinogen deficiency.[30] Nevertheless, others have shown that HF-fed males do not develop hypertension.[35,61] Our results are in line with previous findings by demonstrating that MSEW may exacerbate obesity-induced hypertension through the activation of the angiotensinogen/angiotensin II pathway, as we were able to identify the source for increased circulating angiotensin II associated with angiotensin II–dependent hypertension in female MSEW mice. Moreover, no differences in plasma angiotensin (1-7) concentration was observed between the groups, suggesting that the changes in blood pressure found in MSEW mice are most likely secondary to increased levels of angiotensin II. However, whether MSEW induces sex- and/or depot-specific changes in adipose tissue RAS activation needs further investigation.

Classically, obesity is associated with angiotensin II–dependent hypertension via the activation of sympathetic tone.[62,63] While men and postmenopausal women show a strong correlation between increased body mass index and elevated sympathetic tone, premenopausal women are

mostly protected against increases in nerve activity.[60] In previous studies, we have reported that male rats exposed to maternal separation display increased sympathetic outflow to the kidneys.[64,65] In addition, male MSEW mice fed a HF display higher HR compared with controls.[37] In the current study, we found that female mice fed a HF display increases in HR[30]; however, as reported by others, female MSEW showed similar autonomic function and no changes in HR compared with controls. The normal autonomic function observed in female MSEW mice suggests that other mechanisms, such as metabolic dysfunction, angiotensin II–mediated responses in the vasculature, or changes in water and electrolyte homeostasis, could be responsible for the enhanced obesity-induced hypertension in female MSEW mice fed a HF. Of note, a greater angiotensin II–induced pressor response in untreated MSEW mice with higher endogenous levels of angiotensin II compared with controls may result in vascular desensitization to acute angiotensin II administration. Nevertheless, once the endogenous conversion from angiotensin I to angiotensin II is inhibited, a similar angiotensin II–induced blood pressure between groups supports the notion that MSEW does not enhance this response when angiotensin II levels are similar between groups. Another possible mechanism for the reduced acute blood pressure response in MSEW mice could be sustained by the fact that the ACE inhibition prevents bradykinin actions as a vasodilator.[66–68]

In conclusion, our data extend previous findings in our model by demonstrating that MSEW exacerbates the angiotensinogen/angiotensin II pathway in adipose tissue of female mice fed a HF. This increase in adipose tissue angiotensinogen/angiotensin II production most likely induces elevations in circulating angiotensin II. The upregulation of angiotensinogen/angiotensin II pathway in adipose tissue seems an important mechanism by which female MSEW mice fed a HF develop hypertension. This study highlights the importance of understanding RAS regulation in different contexts (sex, diet, stress), which has the potential to be translated into more personalized treatments for uncontrolled hypertension.

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Disclosures

None.

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Supplemental Material

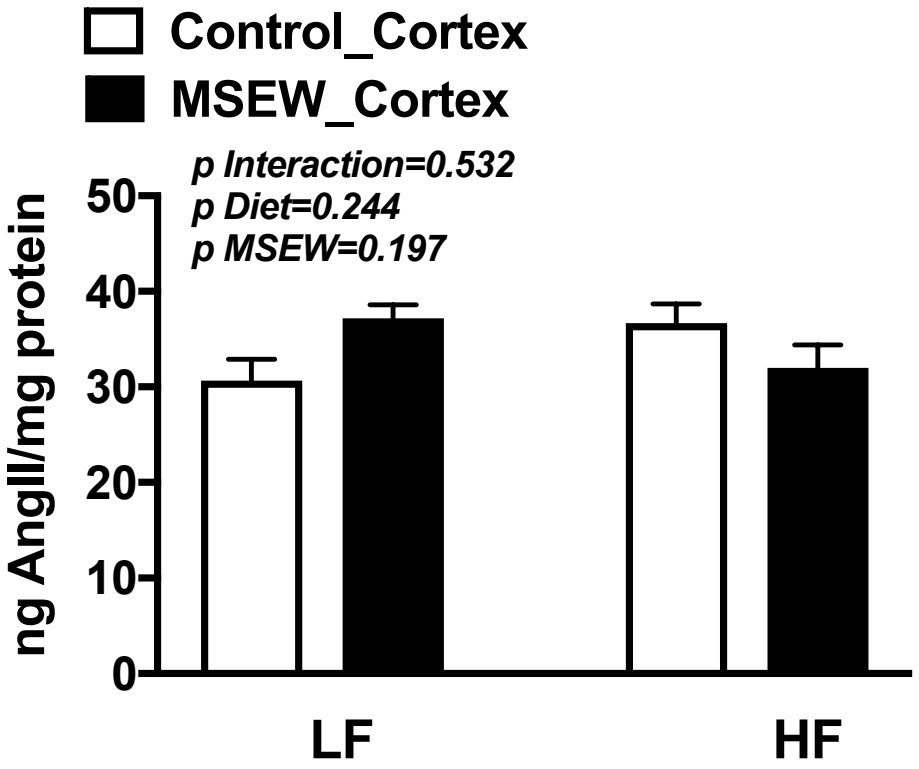

Figure S1. Effect of MSEW in Ang II concentration (Ang II, ng AngII/mg protein) in renal cortex of female control and MSEW mice fed a low fat diet (LF) and high fat diet (HF). Data was analyzed by 2-way ANOVA followed by Bonferroni post-hoc test and reported as mean ± SEM. *: $p<0.05$ vs. Control, #: $p<0.05$ vs. LF; n=8 per group.

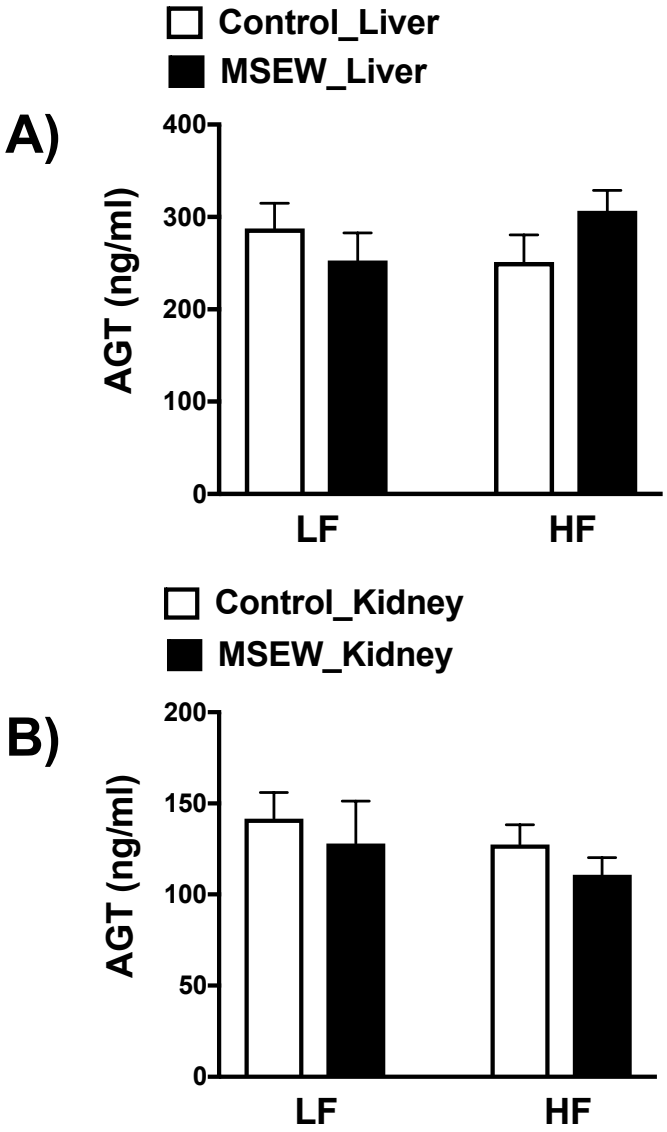

Figure S2. Liver (A) and kidney (B) angiotensinogen content (AGT; ng/ml) in female control (white bars) and MSEW (black bars) mice fed a low fat diet (LF) and high fat diet (HF). AGT content is similar in control and MSEW regardless the diet. n= 6-8 per group.

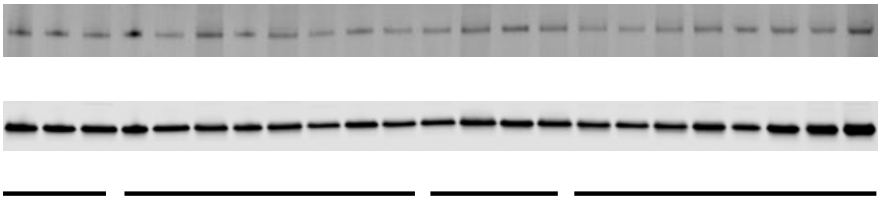

Figure S3. Angiotensin Converting Enzime (ACE) western blot in gonadal white adipose tissue (gWAT) shows no differences between groups.

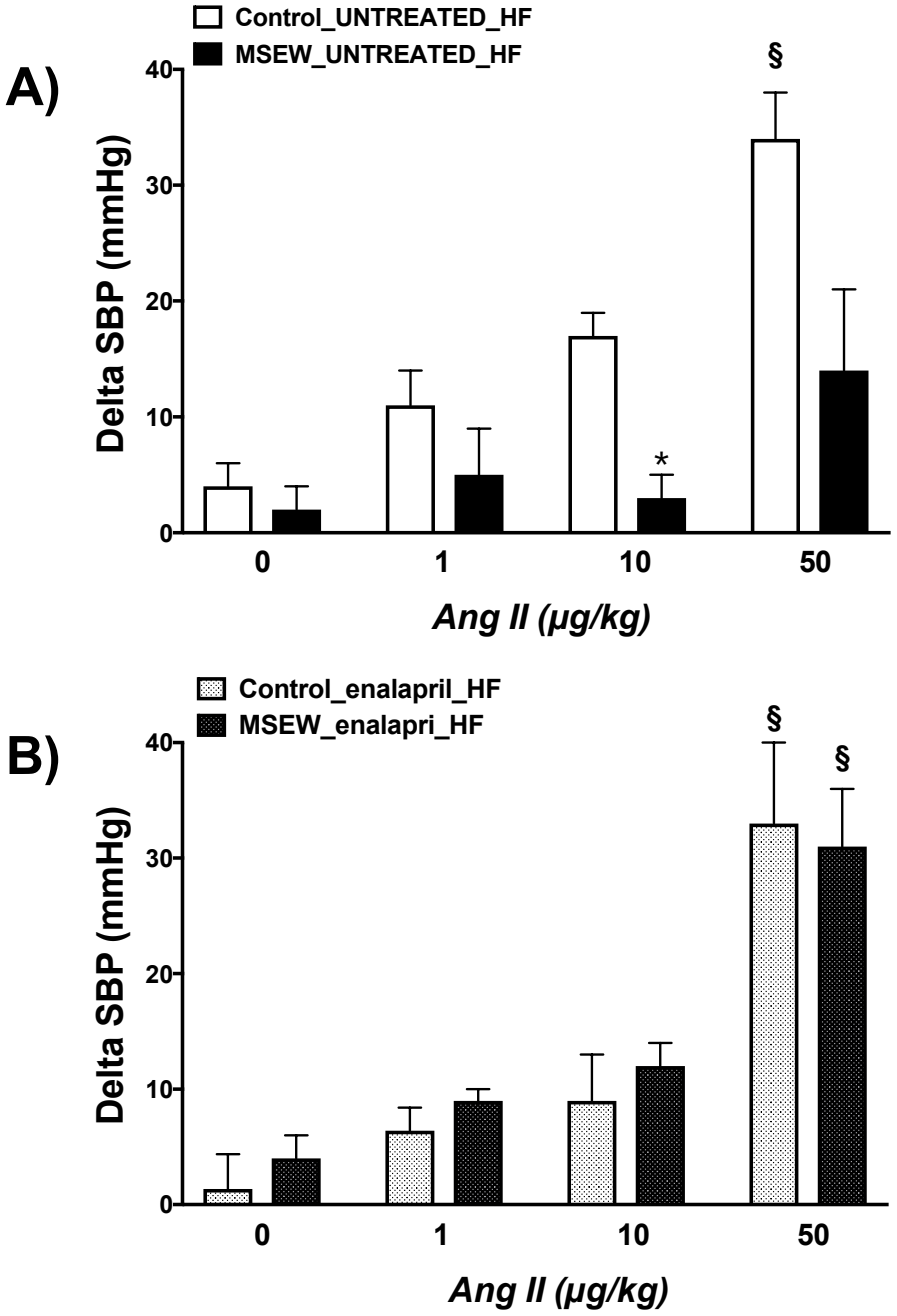

Figure S4. Effect of acute AngII bolus on systolic blood pressure. A) Delta systolic blood pressure changes (delta SBP, mmHg) in untreated and B) enalapril-treated female control (white bars) and MSEW (black bars) mice fed a LF and HF in response Ang II (1, 10 and 50 ng/kg, s.c.). Untreated MSEW mice fed a HF show lower increase in SBP, whereas enalapril treatment abolishes differences in response between control and MSEW mice. Data was analyzed by 1-way repeated measures ANOVA and reported as mean ± SEM. * $p<0.05$ vs. Control, § $p<0.05$ vs. 0, 1 and 10 µg/kg; n=6 per group.