A Model of Neglect During Postnatal Life Heightens Obesity-Induced Hypertension and is Linked to a Greater Metabolic Compromise in Female Mice

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Abstract

Exposure to Early Life Stress (ELS) is associated with behavioral-related alterations, increases in body mass index and higher systolic blood pressure in humans. Postnatal maternal separation and early weaning (MSEW) is a mouse model of neglect characterized by a long-term dysregulation of the neuroendocrine system.

Objectives—Given the contribution of adrenal-derived hormones to the development of obesity, we hypothesized that exposure to MSEW could contribute to worsen the cardiometabolic function in response to chronic high fat diet (HF) feeding by promoting adipose tissue expansion and insulin resistance.

Subjects—MSEW was performed in C57BL/6 mice from postnatal days 2–16 and weaned at postnatal day 17. Undisturbed litters weaned at postnatal day 21 served as the control (C) group. At the weaning day, mice were placed on a low fat diet (LF) or HF for 16 weeks.

Results—When fed a LF, male and female mice exposed to MSEW display similar body weight but increased fat mass compared to controls. However, when fed a HF, only female MSEW mice display increased body weight, fat mass and adipocyte hypertrophy compared with controls. Also, female MSEW mice display evidence of an early onset of cardiometabolic risk factors, including hyperinsulinemia, glucose intolerance and hypercholesterolemia. Yet, both male and female MSEW mice fed a HF show increased blood pressure compared with controls.

Conclusions—This study shows that MSEW promotes a sex-specific dysregulation of the adipose tissue expansion and glucose homeostasis that precedes the development of obesity-induced hypertension.

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Keywords

maternal separation; obesity; hypertension; adipose tissue; sex-differences

Introduction

The prevalence of obesity has increased to epidemic proportions and has become a major heath issue worldwide in children and adults[1–3]. Obesity induces hypertension, a major risk factor for cardiovascular disease (CVD) that affects more than 70 million Americans [4] In addition, metabolic syndrome, also known as syndrome X, refers to a cluster of metabolic conditions that can lead to heart disease. The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) identified six components of the metabolic syndrome that relate to CVD: abdominal obesity, insulin resistance with/without glucose intolerance, atherogenic dyslipidemia, proinflammatory/prothrombotic states and increased blood pressure[5].

To reduce the burden of uncontrolled hypertension morbidity and mortality, it is critical to assess the contribution of less-studied modifiable risk factors. During early life, approximately one third of the population is exposed to a wide range of unpredictable environments, including physical or sexual abuse, neglect, loss of a parent or caregiver, or a natural disaster. Child neglect is the most prevalent form of child maltreatment in the United States, and poses a serious public health concern[6]. Child neglect is frequently defined as the failure of a parent or caregiver to provide proper nutrition, clothing, shelter, medical care, or supervision to the degree that the child's health is at risk. Children who survive such episodes experience long-lasting psychological and behavioral problems[7].

Epidemiological studies identified the exposure to all these forms of early life stress (ELS) as an independent risk factor for type 2 diabetes and ischemic heart disease, in part by increasing body mass index (BMI) and systolic blood pressure (SBP) [8–11]. Findings from the Georgia Stress and Heart Study (GSH) including 213 African Americans and 181 European Americans, revealed that individuals exposed to multiple ELS factors display a greater increase in blood pressure (BP) levels compared to control individuals in a 23-year follow up period [12,13]. Furthermore, the 1958 British Birth Cohort Study including 6,714 members, revealed that metabolic risk was higher among people with psychological distress in childhood only and persist across the life course [14] More recent studies highlighted that when the environment is harsh and unpredictable, a fast life history strategy is adaptive. That is, in these environments, somatic efforts are unsuitable. However, fast life-history strategies has been linked to impulsivity and a focus on short-term goals as a causal factor for high obesity risk [15]. A role for maternal care during early life has been closely observed. In fact, poor quality of the early maternal-child relationship was associated with a higher prevalence of adolescent obesity[16]. For instance, in a 3.5-year prospective study conducted in 6-month old babies, a significant association was demonstrated between low maternal sensitivity (i.e. timely and appropriate response on the part of the mother towards the cues of her infant) and high body mass indices.[17]

Although the prevalence of hypertension is higher in men than in age-matched, pre-menopausal women [18], population-based studies during the last decade show that obesity affects women more than age-matched men, also identified as the cause of the 3-fold increase in the risk for hypertension in premenopausal women[13–15]. Also, stress-related disorders (e.g., depression and anxiety), obesity and hypertension are comorbidities observed more frequently in women beginning in adolescence [19–22]. Importantly, a growing body of evidence suggests that long-term effects of ELS may impact women's susceptibility to gain weight and develop insulin resistance in a greater fashion than men [8,23]. Yet, an established experimental paradigm to determine the sex-specific effects of ELS on the origins of obesity-induced hypertension is missing, demanding the validation of models to decipher the causative mechanisms of this relationship.

A widely-used animal model for the investigation of molecular and behavioral responses to ELS is the maternal separation paradigm. Developed as a rat model, pups are subjected to daily maternal separation (180 min=MS180) from postnatal days 2–14 [24–27]. Efforts have been made to adapt the paradigm to mice in order to study in-depth molecular pathways using transgenic approaches. However, attempts to replicate the MS180 phenotype in mice have displayed mixed results [28–33]. The lack of consistency with regard to maternal separation in mice seems to be driven by at least two components: both the duration of separation and the quality of maternal care provided to the pups upon being returned to the nest. For these reasons, Simens' group developed a model called Maternal Separation with Early Weaning (MSEW), which includes separation periods that occur over a broad range of postnatal ages and an additional component of early weaning [34] As developed, MSEW is considered a mouse model of neglect that has been associated with the overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, changes in the brain architecture and misprogramming of the vascular function in mice fed a standard chow diet [34–37]. To date, this paradigm may serve as a unique tool to decipher the link between ELS and CVD risk. In this study, we combine MSEW with an obesogenic diet starting at weaning to model the interplay between these two environmental stressors. Therefore, we hypothesized that MSEW will worsen the development of diet-induced obesity and hypertension via increases in: 1) fat mass and adipocyte hypertrophy; 2) cardiometabolic risk factors (e.g. plasma insulin, leptin, lipids and glucose intolerance); and 3) blood pressure and heart rate after 16 weeks of high fat diet (HF) feeding. Furthermore, we performed this study in male and female mice to investigate a potential sex-specific cardiometabolic susceptibility secondary to MSEW exposure.

Methods

Animal model

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and 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 and housed in a pathogen-free environment with constant temperature and humidity with a 14:10 hour light: dark cycle[38]. Maternal separation with early weaning (MSEW) protocol was performed in C57BL/6 mice as

follows: pups were separated from their mothers by transferring the pups to a clean cage in an incubator ($30 \pm 1°$ C) for 4 hours from postnatal day 2 to day 5 and for 8 hours from postnatal day 6 to 16 of life with early weaning at postnatal day 17. Normally reared, non-handled litters remained with their mother and served as the control group and were weaned at postnatal day 21. For the LF-fed groups, we used 9 control litters and 11 MSEW litters. For the HF-fed groups, we used 12 control litters and 14 MSEW litters. Litters were included when size ranged from 6 to 8 pups and a different diet was randomly assigned.

Experimental Design

Dams were fed a regular chow diet (Teklad 8604, Madison, WI). Upon weaning, offspring were fed a LF (10% kcal from fat, F6256, BioServ, Frenchtown, NJ) or a HF (60% kcal from fat, F2685, BioServ, Frenchtown, NJ) for 16 weeks. All the experiments were conducted in both male and female mice. Body weight was measured weekly and body composition was measured monthly by an Echo Magnetic resonance imaging system (Echo-MRI; Echo Medical Systems, Houston, TX). At week 13, mice were fasted for 16 hours to perform an oral glucose tolerance test (OGTT). After an oral gavage of D-glucose (2 g/kg body weight)[39], blood glucose measurements were taken via tail prick at 0, 15, 30, 60, and 120 minutes using a glucometer (Accu-check, Roche, Germany). Area under the curve was calculated using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). At weeks 7 and 14, food intake was measured in individually-housed mice for 5 consecutive days. By the end of week 14, a subset of mice was implanted with radiotelemetry for the monitoring of blood pressure, heart rate and locomotor activity. At the end of the study, animals were anesthetized with ketamine/xylazine (100/10 mg/kg, IP) for exsanguination by cardiac puncture and tissue harvesting. Liver and white adipose tissue (WAT) were collected and snap frozen for mRNA quantification or fixed in 10% buffered formalin for histological analysis.

Histological analysis of adipose tissue morphology

The gonadal (epididymal or periovaric) WAT depots were collected in 10% buffered formalin, fixed for 24 hours, and paraffin-embedded. Following, 5-μm sections were cut and stained with hematoxylin and eosin. Images of slides were obtained at ×10 magnification. Using the "detect edges," image threshold, and object count features of NIS Elements software (Nikon Instruments, Inc., Tokyo, Japan), the area of each adipocyte and cell number within a 1500×1500 μm^2 measurement frame were quantified. Adipocyte size and number were quantified on two measurement frames within each section of adipose tissue (n=3 sections/mice) from mice in each group. The blinded quantification of the adipocyte cross-sectional size was expressed as ratio of the MSEW /Control.

Quantitative real time PCR

RNA was extracted from dissected gonadal WAT and liver using Aurum total RNA mini kit (Bio-Rad, Hercules, CA) as previously described [40]. Briefly, total mRNA was extracted from tissues using the TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The mRNA concentrations were quantified via the NanoDrop 2000 spectrophotometer (Applied Biosystems, Waltham, MA) and reverse transcription was performed using the iScript cDNA Synthesis kit (Bio-Rad). The levels of

mRNA expression were assessed by quantitative real-time PCR using a CFX 96 Touch Detection System (Bio-Rad) and iTaq Univer SYBR Green supermix (Bio-Rad). Data analysis was performed using the relative quantification method ($2^{-\Delta Ct}$), in which relative mRNA expression for target mRNAs was compared to a constitutively expressed gene (i.e., 18S) within mRNA samples from tissues. Primer sequences for SYBR Green chemistry were designed using the Primer Express Software 3.0 for RT-PCR (Applied Biosystems).

Plasma risk factors for obesity and insulin resistance

Plasma was collected using EDTA 7.5% solution. Leptin, insulin, corticosterone and aldosterone EIA kits (Cayman, Ann Arbor, MI) and adiponectin (Invitrogen, Waltham, MA) were performed following manufacturer's specifications. Serum cholesterol and plasma triglyceride were determined by an enzymatic kit (Wako Diagnostics, Richmond, VA).

Blood pressure measurement using radiotelemetry

Blood pressure was measured by radiotelemetry during week 16 as previously described [41]. Briefly, anesthetized (isoflurane) male and female mice fed a HF were implanted with carotid artery catheters and telemetry devices (Data Sciences, Inc., St. Paul, MN) during week 15 of HF feeding, allowed 10 days to recover. Then, mean arterial pressure (MAP), heart rate (HR) and locomotor activity within the cage were continuously recorded during 3 consecutive days using the Dataquest ART Acquisition program (Data Sciences International, St. Paul, MN) and expressed in 24 hour averages.

Statistical Analysis

Prior to analysis, all observations were averaged within litters, and analyses were performed with litters as experimental units. All data are presented as mean±SEM. Differences in means among groups and treatments were compared by 2-way ANOVA to assess the effect of diet and separation in each sex. To adjust for the false positive rate due to multiple testing we have used Benjamini-Hochberg adjustment. Fisher's LSD post-hoc tests were calculated in all significant ANOVAs.

Results

Supplementary information is available at IJO's website at the end of the article and before the references.

Effect of MSEW on body composition, organ weight and food intake

Body weight was not different in MSEW and control weanlings at postnatal day 21 (Fig. S1). Likewise, adult male and female MSEW mice fed a LF displayed comparable body weight (Fig. 1) and tissue weights (Table S1) but increased adiposity (Fig. 1B and 1D) after a 16-week period. In HF-fed males, body weight (Fig. 1A), fat mass (Fig. 1B, top) and lean mass (Fig. 1B, bottom) percentages were not statistically different between MSEW and control mice. However, HF-fed female MSEW mice showed increased body weight (Fig. 1C), fat mass (Fig. 1D, top) and reduced lean mass (Fig. 1D, bottom) compared to controls. In addition, HF diet also increased liver, gWAT and scWAT depots weights in female MSEW

compared to controls (Table S1). These increases in body weight and adiposity in female MSEW mice seem independent of significant variations in food intake (Table S2).

Effect of MSEW on adipose tissue morphology

Male mice showed no influence of MSEW on gWAT morphology (Fig. 2A), as adipocyte diameter and area (Fig. 2B, top) were similar between groups. The log (MSEW/ control frequency) ratio revealed that male mice have a similar adipocyte diameter regardless of separation or diet factors (Fig. 2B, bottom). However, adipocyte size was increased in female MSEW mice fed a HF (Fig. 2C). Specifically, female MSEW mice displayed hypertrophy of adipocytes, increases in cell diameter and reduced cell number compared to control mice (Fig. 2D, top). Also, MSEW displayed increased frequency of the larger diameter adipocytes (Fig. 2D, bottom). Yet, scWAT adipocytes area and diameter were similar in MSEW and control female mice fed a HF (Fig. S2); however, adipocytes from 3000–5000 μm area were bigger in MSEW mice compared to controls. This data suggests that gWAT expansion is an important contributor for the greater fat mass observed in HF-fed female MSEW mice. Because of these changes in adipocyte morphology observed in female mice, we analyzed body fat distribution by MRI (Fig. S3). Female MSEW and control mice displayed similar subcutaneous adipose tissue (SAT) volume (Fig. S3C); however, visceral adipose tissue (VAT) was increased in female MSEW fed compared to controls regardless of diet (Fig. S3D).

Effect of MSEW on adipose tissue gene expression

MSEW increased 11βHSD-1 mRNA abundance in gWAT only in male mice fed a LF (Fig. 3A). Exposure to MSEW did not influence GR mRNA expression in mice fed a LF, while increased it in all groups of HF-fed mice (Fig. 3B). Conversely, MR expression was increased in male and female MSEW mice fed a LF; however, only female MSEW mice fed a HF showed further increases in MR expression compared with controls. We also determined the mRNA abundance of PPARg, a master regulator of adipocyte differentiation, which was increased only in LF-fed female MSEW mice compared with controls but not in HF-fed mice. Finally, no significant effects of separation or diet were found in IL-6 and TNF-α mRNA expression in male mice, while MSEW increased these inflammatory markers only in female mice fed a HF. The mRNA levels of the housekeeper gene 18S in all groups are shown in Table S3, and the sequence for RT-PCR primers is listed in Table S4.

Effect of MSEW on plasma risk factors for obesity and insulin resistance

Overall, MSEW did not influence the plasma metabolic profile in mice fed a LF diet. While HF-fed male MSEW and control mice showed similar levels of plasma insulin (Fig. 4A), female MSEW mice displayed hyperinsulinemia compared with controls (Fig. 4D). HF induced comparable increases in plasma leptin in both groups and did not affect adiponectin. Furthermore, we determined the effect of MSEW on the glucose disposal.

Fasting glucose was not different between groups, though there was a trend for elevated fasting glucose with HF in both male and female MSEW mice (Table S5). OGTT was increased similarly in male MSEW and control mice fed a HF (Fig. 4B). However, female MSEW showed greater impairments in glucose tolerance in both LF and HF compared with

control female mice (Fig. 4E). The atherogenic profile revealed that HF induced similar increases in plasma cholesterol in MSEW and control males (Fig.5C). However, in females, HF increased triglycerides similarly in both groups but significantly increased cholesterol levels in MSEW compared with controls (Fig. 4F).

Effect of MSEW on adrenal cortex-derived hormones

Table 1 shows similar plasma levels of corticosterone and aldosterone in MSEW and control mice fed a LF diet. HF-induced increases in these hormones in both sexes, although there were no differences between MSEW and control mice.

Effect of MSEW on obesity-induced increases in blood pressure

MSEW increased 24 hour-MAP and SBP in male and female mice fed a HF (Fig. 5A and 5D). No differences were observed in DBP due to separation in either sexes. MSEW increased HR only in male mice (Fig. 5B and 5E), while no significant differences in either sex were observed in locomotor activity (Fig. 5C and 5F) compared with controls.

DISCUSSION

This study reveals that MSEW, a mouse model of neglect, heightens obesity-induced hypertension in male and female mice fed a HF since weanlings. Despite significant increases in blood pressure in both sexes, hypertension was accompanied by increased HR only in male mice exposed to MSEW. Conversely, HF induced greater percentages of fat mass, adipocyte hypertrophy, insulin resistance and hypercholesterolemia in female MSEW mice compared to female controls. Taken together, our study highlights that MSEW serves as a unique model to decipher sex-specific mechanisms linking ELS and cardiovascular risk in the onset of metabolic disease.

ELS is defined by exposure to a wide range of adverse childhood experiences such as abuse, neglect or loss during the first decade of life that imparts negative effects on health outcomes. Specifically, epidemiological studies indicate that adults exposed to ELS develop several risk factors for cardiovascular and metabolic disease including elevated systolic blood pressure (SBP), BMI, waist circumference, and resting HR [11,13,14,42]. Moreover, ELS has a positive correlation with the clustering of metabolic risk biomarkers such as higher circulating levels of leptin and the acute phase proinflammatory mediator, C reactive protein (CRP), as well as elevations in plasma glucose and insulin [43–45]. Among the mechanisms postulated, it was proposed that children that underwent ELS are at greater risk for negative affect and internalizing disorders such as depression and anxiety [46–48], which may contribute to behavioral eating disorders including unhealthy dietary choices [49,50,51]. These alterations have been often linked to the early life dysregulation of the sympathetic nerve activity (SNA) and/or the HPA axis [52–54]. Despite most of these studies have not been designed to test sex-specific effects on the cardiovascular and metabolic risk, a growing body of evidence shows that long-term effects of ELS may impact women's susceptibility to gain weight and develop insulin resistance at a greater fashion [23,55–57]; however, the potential origins for this predisposition remain unclear.

Maternal separation has been shown to induce detrimental effects on behavioral and neuroendocrine responses that mimic several aspects of ELS-induced derangements in humans[24–27]. Efforts have been made to adapt the paradigm to mice given the advances in murine studies since the C57Bl/6 mouse genome has been sequenced and satisfactorily annotated. However, anxiety and stress response in maternal separation in mice has been reported to be increased [28,29], decreased [30] or unchanged [31]. A more comprehensive study in mice revealed that levels of maternal care (i.e. licking, nursing, covering, pup handling, and nesting) increase as a function of MS180 immediately after daily maternal separation, but long-term care remains unchanged, showing that C57Bl/6 mice are resilient to maternal separation, exhibiting subtle decreases in anxiety and unchanged stress-induced corticosterone response as adults, irrespective of separation duration[58]. Hence, the MSEW model has been generated with the goal to replicate a consistent phenotype. As adult, mice exposed to MSEW display alterations across several behavioral domains, increases in hyperactivity, anxiety, and depressive- like behavior [34,35,37].

To note, studies using maternal separation paradigms have been strongly focused on the behavioral aspects of the phenotype, reporting the absence in changes of pup mortality or morbidity, weight, or metabolic derangements. Accordingly, we found that neonates exposed to MSEW show normal BW compared with control mice at postnatal day 21. Thus, MSEW does not induce a catch-up growth effect in the pups and weanlings, which is a hallmark of low birth weight models associated with greater cardiovascular and metabolic risk later in life [59–61]. Nevertheless, the long-term consequences in the cardiometabolic responses have been disregarded. We speculate that MSEW could not be restricted to psychological/ psychosocial stress only, and may contribute with early metabolic disturbances that predispose to the development of obesity. Yet, the molecular mechanisms involved in such hypotheses have not been rigorously tested. Therefore, we cannot rule out the contribution of prolonged periods of food constraint with endocrine and metabolic alterations influencing the adipose tissue plasticity and glucose homeostasis during early life. While there is no direct evidence to support this association, the assessment of the body composition and metabolic function in adult mice exposed to models of maternal separation involving shorter periods of time, such as MS180, could be helpful to tease out the impact of the prolonged separation procedures combined with early weaning per se.

To investigate whether postnatal MSEW worsens the cardiometabolic function, we subjected these mice to two dietary conditions. First, we determined the long-term effect of MSEW on body composition and metabolic responses in mice fed a LF. MSEW did not influence BW, organ weight and adipose tissue morphology; however, MSEW increased the percentage of fat mass in both male and females. This data suggest that increased adiposity may predispose these mice to develop obesity-hypertension later in life. Thus, investigating the effect of stress (or glucocorticoids) early on adipocyte differentiation may provide some insights on the mechanisms by which MSEW increases fat mass in these mice. Second, we challenged MSEW to a secondary stressor (or "second hit") by feeding them a HF starting at weaning. HF increased BP in male and female exposed to MSEW; however, adipocyte hypertrophy, impaired glucose disposal, and hypercholesterolemia were heightened only in female MSEW mice compared to controls. Further, LF-fed female MSEW mice showed an early onset of cardiometabolic risk including greater visceral fat deposition and impaired

glucose tolerance. Taken together, these data indicate that the obesogenic diet induces a metabolic compromise in female MSEW mice that could play an important role in the lack of protective factors against cardiovascular risk.

Increased plasma glucocorticoids are a hallmark of obesity-associated metabolic derangements [62]. However, despite the effect of HF, MSEW did not increase plasma corticosterone. Circulating glucocorticoids are tightly controlled by the activation of the HPA axis; however, tissue-specific availability is regulated by the receptor expression, affinity, and alterations in glucocorticoid metabolism and clearance [63–65]. Intracellular levels of glucocorticoids are influenced by the enzyme 11β-HSD1, which converts inactive cortisone to its active form corticosterone [66]. Thus, at the tissue level, the relative contribution of glucocorticoids and mineralocorticoid are influenced by the relative abundance of MR and GR, circulating levels of glucocorticoids and aldosterone, and local conversion of cortisone-cortisol by HSD1 and HSD2 [67]. Dasarzens and Farasse have reported that the adipocyte-specific deletion of GR did not affect body weight gain or adipose tissue differentiation and distribution; however, the lack of GR in adipocyte was associated with higher pro-inflammatory genes. They also showed that adipose tissue GR ablation did not correlate with insulin resistance or dyslipidemia, but was associated with disturbed glucose tolerance, supporting the notion that GR deletion may impair adipose tissue function but not its expansion during a high calorie diet [68]. Recent studies demonstrate that MR (NR3C2) is expressed in adipose tissue and is implicated in both adipogenesis and adipose endocrine function [65,69]. Since the MR binds to cortisol with 10-fold higher affinity than GR [70], high local concentration of cortisol within adipose tissue may activate both GR and MR. MR activation increases proinflammatory adipokine expression such as interleukin-6 (IL-6) [71]. Conversely, MR blockade reduces inflammation in adipose tissue and increases the expression of adiponectin in heart and adipose tissue of obese, diabetic mice [72]. Therefore, increased fat mass in LF-fed MSEW mice could be linked with a greater effect of mineralocorticoids at the tissue level secondary to excess of glucocorticoids. Interestingly, HF-induced MR mRNA upregulation was further augmented only in tissue from female MSEW mice, suggesting that the local actions of mineralocorticoids could be implicated in the mechanism by which MSEW promotes adipose tissue expansion in a sex-specific manner.

Given the important contribution of the liver to the glucocorticoid metabolism [73], we also determined the effect of MSEW on the hepatic expression of the enzyme 11β-HSD1. While HF-fed female MSEW mice showed a greater hepatic pro-adipogenic capacity (Fig. S4), normal hepatic gene expression in mice fed a LF supports the concept that increased adiposity could precede the metabolic derangements in these mice. Taken together, our study is consistent with the idea that MSEW-induced HPA dysregulation at tissue level could play an important role in promoting adiposity and metabolic dysfunction, particularly in female mice fed a HF.

It has been shown that diet-induced obesity starting at weaning upregulates systemic and adipose tissue glucocorticoid metabolism [74]; however, a handful of studies have challenged maternally separated rodents to a obesogenic diet [75–77]. Recently, our laboratory has shown that female rats exposed to maternal separation (3 hours a day, postnatal days 2–14) respond

to a chronic HF by increasing body weight and fat mass compared with their control littermates [40]. Nonetheless, this increase in adiposity was not associated with elevated blood pressure [40]. Elevated levels of glucocorticoids during the postnatal "stress hyporesponsive period", which takes place during the first two weeks in life in rodents, has been proposed as causal factor for the long-lasting increased HPA axis sensitivity to secondary stressors [24,36,78]. Interestingly, we showed that the pharmacological inhibition of the corticosterone synthase during postnatal life abrogated the heightened effects of diet-induced obesity in female rats, suggesting that postnatal exposure to corticosterone appears to be a key event associated with the programming of the adipose tissue plasticity in females [40].

In summary, we have shown that MSEW serves as a unique approach for the study of the ELS-induced sexual dimorphism on the cardiovascular system, particularly in the onset of metabolic disease. Our previous work in a rat model of maternal separation [40] and the present study performed in a mouse model of neglect, demonstrate that female rodents exposed to postnatal stress display a lack of protective factors against diet-induced obesity, which may predispose them to develop worsened cardiovascular outcomes. As women show greater incidence of depression, stress and cardiovascular risk comorbidities [19,21,79], early life-induced alterations in the neuroendocrine mechanisms interplaying with cardiovascular pathways may place females at higher risk for these comorbidities during adult life. Our study contributes to the advancement of the Developmental Origins of Health and Disease (DOHaD) field by validating a paradigm linking ELS and cardiovascular risk, with the primary goal to provoke further clinical and experimental studies on this important translational research topic. Thus, interventions aimed at improving the quality of maternal-child interactions should consider assessing effects on children's weight and examining potential mechanisms involving stress response and emotion regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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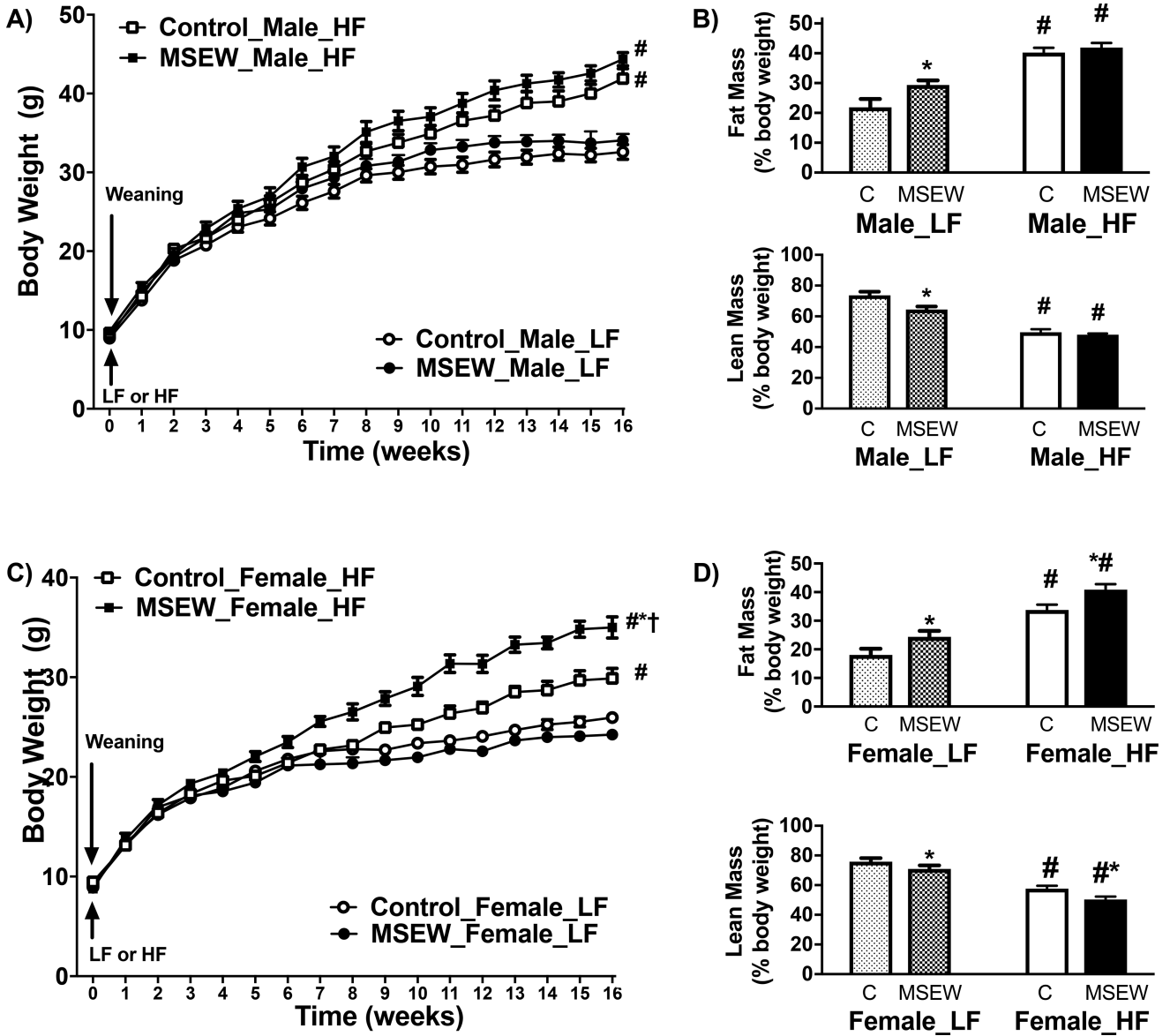
Figure 1.
Effect of MSEW in body weight (BW) and body composition after 16 weeks on LF or HF diet initiated at weaning: A) BW and B) body composition in male mice; C) BW and D) body composition in female mice. MSEW male LF (n=9), control male LF (n=9), MSEW male HF (n=13), control male HF (n=11), MSEW female LF (n=11), control female LF (n=9), MSEW female HF (n=14), control female HF (n=12).
Test between subjects Body weight
Male mice: Diet= F ratio (107), Prob>F (<0.0001); Separation= F ratio (3.9), Prob>F (0.053); Diet*Separation= F ratio (0.36), Pro>F (0.54). Female mice: Diet= F ratio (84), Prob>F (<0.0001); Separation= F ratio (5.8), Prob>F (<0.002); Diet*Separation= F ratio (7.4), Pro>F (<0.01). * P separation, # P diet, †P interaction.
Test between subjects % fat mass

Male mice: Diet= F ratio (101), Prob>F (<0.0001); Separation= F ratio (12.8), Prob>F (0.001); Diet*Separation= F ratio (6.6), Pro>F (0.01). Female mice: Diet= F ratio (64), Prob>F (<0.0001); Separation= F ratio (11.2), Prob>F (<0.002); Diet*Separation= F ratio (0.03), Pro>F (0.85).
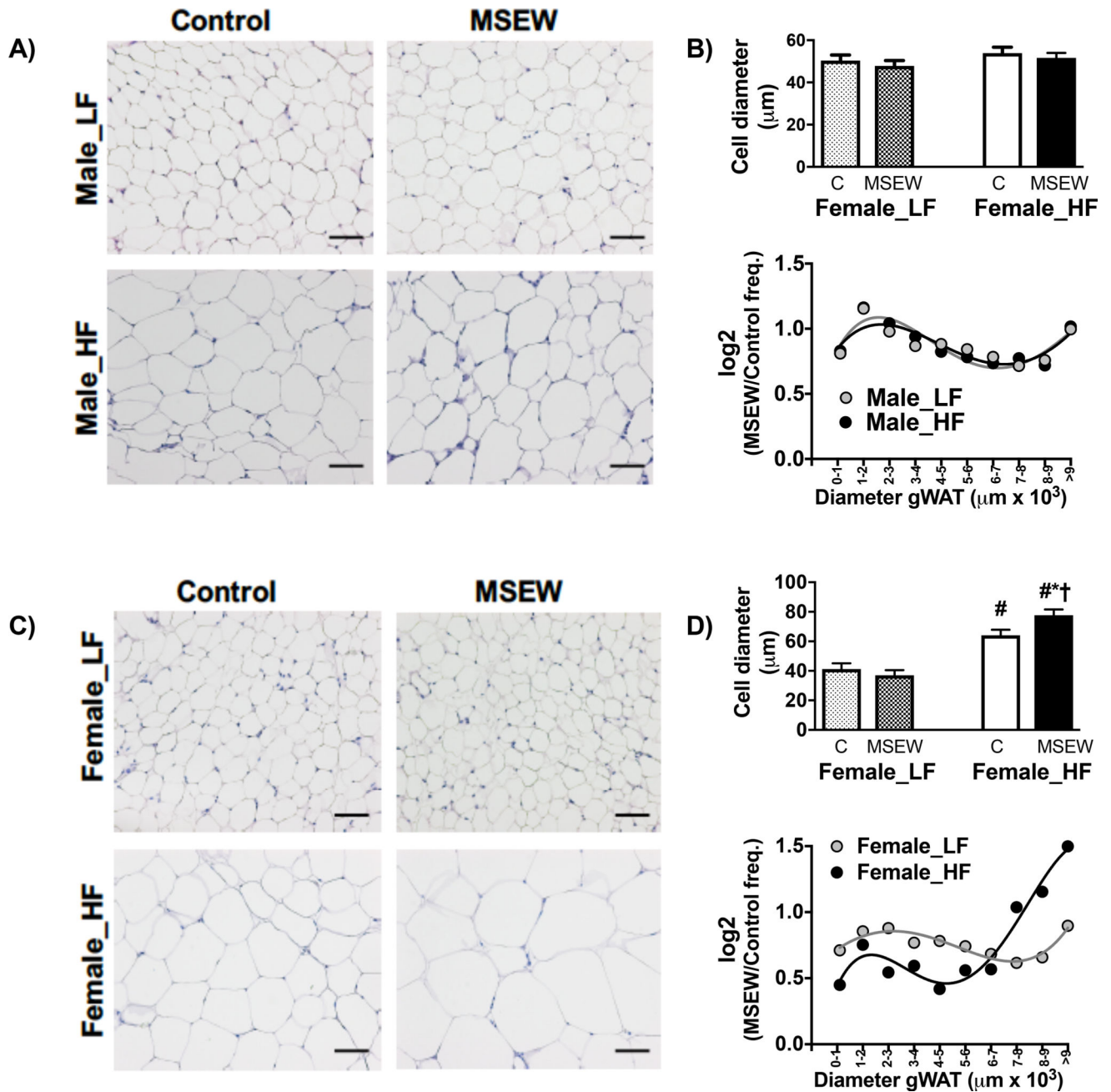
Figure 2.

Effect of MSEW on white adipose tissue morphology after 16 weeks on LF or HF diet initiated at weaning: A) adipocyte morphology and B) adipocyte diameter histogram in male mice; C) adipocyte morphology and B) adipocyte diameter histogram in female mice. N=4–5 per group. * P separation, # P diet, †P interaction.

Test between subjects adipocyte hypertrophy:

Male mice: Diet= Prob>F (NS); Separation= Prob>F (NS); Diet*Separation= Pro>F (NS).

Female mice: Diet= F ratio (71), Prob>F (<0.0001); Separation= F ratio (1.6), Prob>F (0.22); Diet*Separation= F ratio (5.7), Pro>F (0.03).
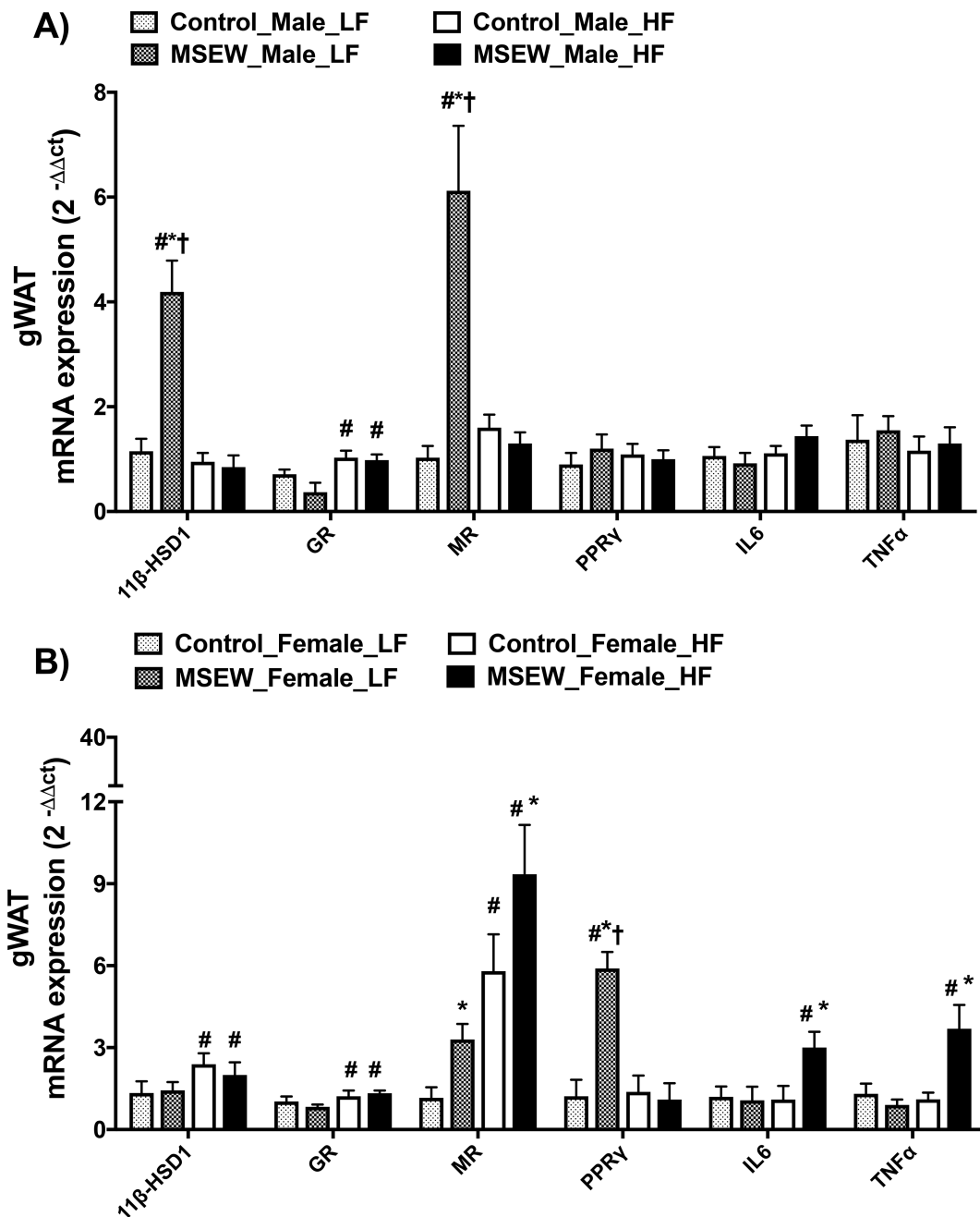
Figure 3.
Effect of MSEW on gonadal WAT (gWAT) mRNA expression after 16 weeks on LF or HF initiated at weaning in A) male and B) female mice. N=5–8 per group.

* P separation, # P diet, †P interaction.

Test between subjects gWAT 11bHSD1 gene expression

Male mice: Diet= F ratio (26), Prob>F (<0.0001); Separation= F ratio (17.7), Prob>F (0.0004); Diet*Separation= F ratio (21), Pro>F (0.0002). Female mice: Diet= F ratio (5), Prob>F (<0.03); Separation= F ratio (0.33), Prob>F (0.57); Diet*Separation= F ratio (0.15), Pro>F (0.69).

Test between subjects gWAT MR gene expression

Male mice: Diet= F ratio (13), Prob>F (0.002); Separation= F ratio (16.4), Prob>F (0.0007); Diet*Separation= F ratio (21), Pro>F (0.0002). Female mice: Diet= F ratio (36), Prob>F (<0.0001); Separation= F ratio (13.5), Prob>F (<0.001); Diet*Separation= F ratio (0.87), Pro>F (0.35).

Test between subjects gWAT PPARg gene expression

Male mice: Diet= Prob>F (NS); Separation= Prob>F (NS); Diet*Separation= Pro>F (NS). Female mice: Diet= F ratio (5.5), Prob>F (0.02); Separation= F ratio (5), Prob>F (0.03); Diet*Separation= F ratio (6.3), Pro>F (0.02).
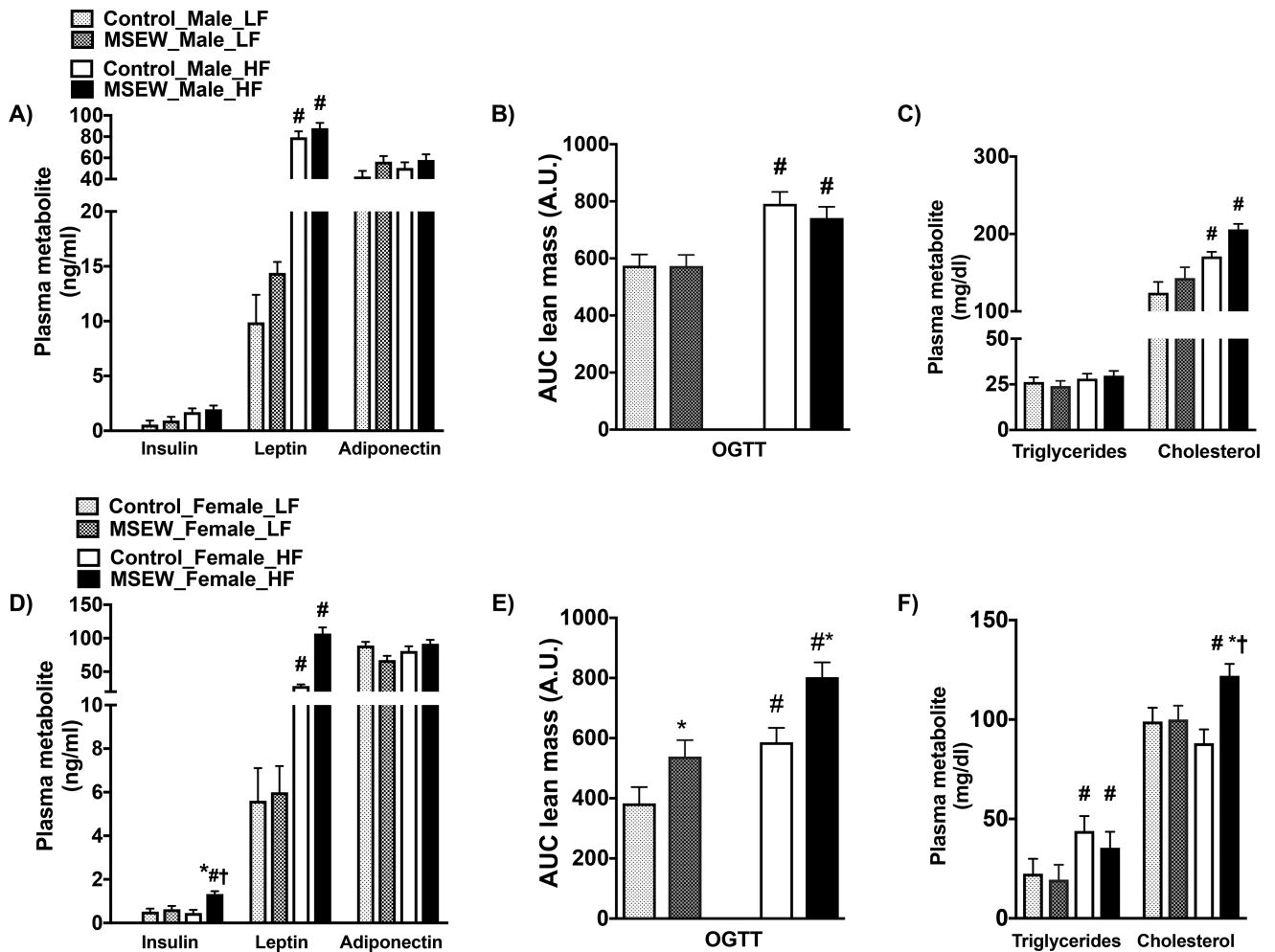
Figure 4.
Effect of MSEW on the plasma metabolic profile after 16 weeks on HF initiated at weaning in males and female mice. Insulin, leptin and adiponectin in A) male and D) female mice. Oral glucose tolerance test (OGTT) in B) male and E) female mice. Plasma triglycerides and cholesterol in C) male and F) female mice. N=6–12 per group.

* P separation, # P diet, †P interaction.

Test between subjects insulin

Male mice: Diet= Prob>F (NS); Separation= Prob>F (NS); Diet*Separation= Pro>F (NS).

Female mice: Diet= F ratio (5.2), Prob>F (<0.03); Separation= F ratio (13.5), Prob>F (<0.01); Diet*Separation= F ratio (0.03), Pro>F (0.85).

Test between subjects OGTT

Male mice: Diet= F ratio (23), Prob>F (<0.0001); Separation= F ratio (0.40), Prob>F (0.53); Diet*Separation= F ratio (0.37), Pro>F (0.55). Female mice: Diet= F ratio (20), Prob>F (<0.0001); Separation= F ratio (12.8), Prob>F (<0.001); Diet*Separation= F ratio (0.33), Pro>F (0.56).

Test between subjects Cholesterol

Male mice: Diet= F ratio (17), Prob>F (0.0003); Separation= F ratio (4.2), Prob>F (0.051); Diet*Separation= F ratio (0.38), Pro>F (0.54). Female mice: Diet= F ratio (0.64), Prob>F

(0.43); Separation= F ratio (6.6), Prob>F (<0.02); Diet*Separation= F ratio (5.5), Pro>F (0.03).
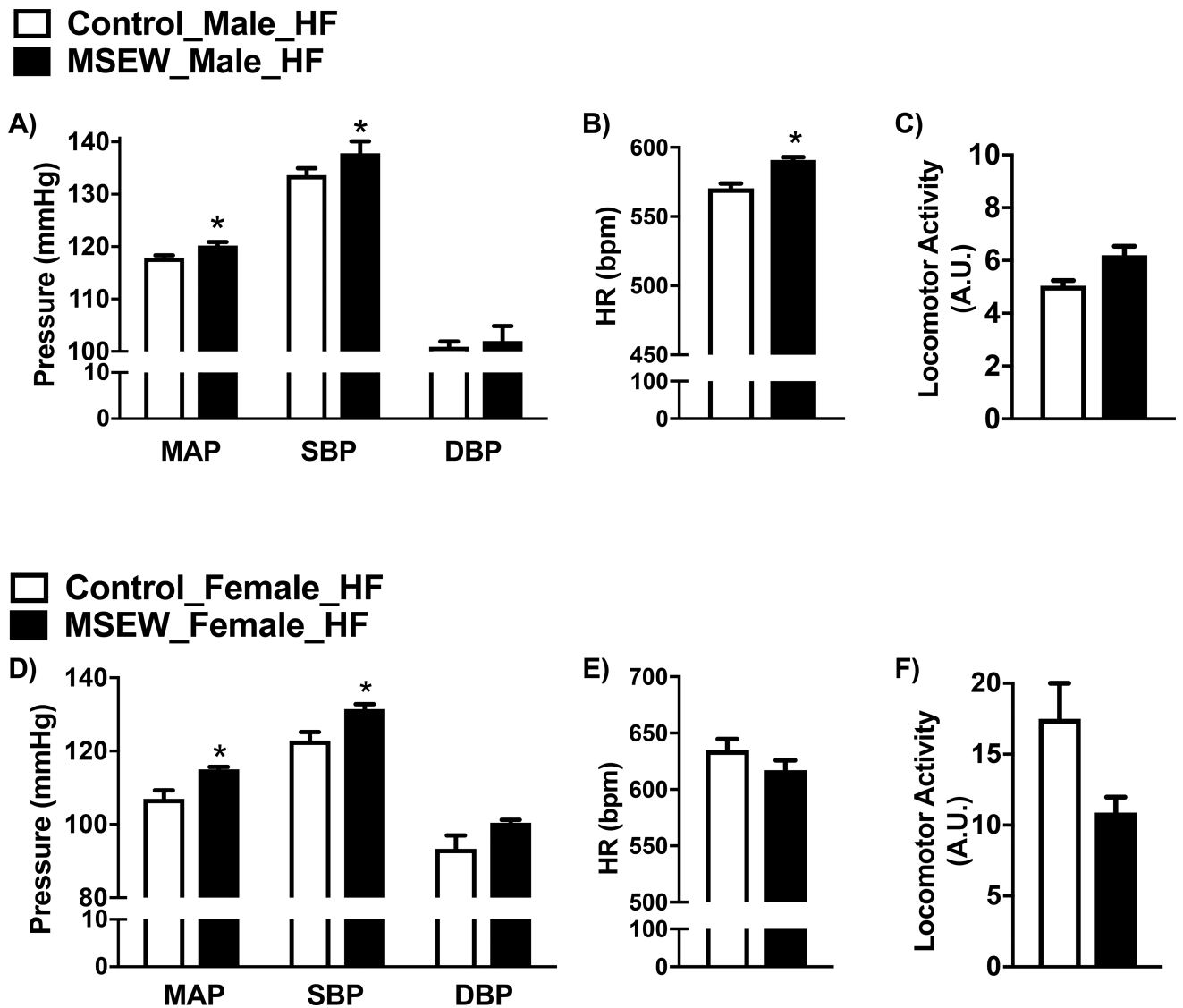
Figure 5.
Effect of MSEW on 24hr hemodynamic parameters after 16 weeks on HF initiated at weaning. A) mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP); B) heart rate (HR) and C) locomotor activity in male mice. D) mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP), E) heart rate (HR) and F) locomotor activity in female mice. N=4–5 per group. * P <0.05 vs. control.

Table 1

Adrenal-derived hormones in male and female mice fed 16 weeks on a LF or HF diet. N=6–9 per group. # P <0.05 vs. LF.

		Control LF	MSEW LF	Control HF	MSEW HF
Corticosterone (ng/ml)	**Male**	53±12	65±8	82.1±22.0	114.6±31.1#
	Female	49±9	42±11	72.9±15.9	139.4±28.5#
Aldosterone (ng/ml)	**Male**	8.9±2.5	11.9±2.3	129.1±25.11 #	132.0±21.5#
	Female	63.9±27	101.8±21	333.7±70.7#	447.6±65.7#