Prenatal Stress or High Fat Diet increases Susceptibility to Diet-Induced Obesity in Rat Offspring.

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ABSTRACT

Objective – Perturbations to the prenatal environment has been associated with the development of adult chronic disease, findings that gave rise to the “Barker Hypothesis” or the “developmental origins of adult disease” concept. In this study we used an animal model to determine the metabolic consequences of maternal prenatal stress and high fat feeding on the developing offspring.

Research Design and Methods – Pregnant female Sprague-Dawley rats were maintained on standard chow (CHOW) or 60% high fat (HF) diet throughout gestation and lactation. Half of each group were exposed to a novel variable stress paradigm (STRESS) during the third week of gestation while control dams were left undisturbed (CON). Body weight, body composition, glucose tolerance and endocrine parameters were measured in offspring through early adulthood.

Results – Male and female pups from dams that experienced prenatal stress and/or were on HF diet weighed more beginning on postnatal day 7 compared to CHOW-CON. Access to HF diet at weaning enhanced increased the body weight effect through early adulthood and was attributable to greater adiposity. Pups weaned onto CHOW diet showed no significant difference in glucose clearance or insulin secretion. However, pups weaned onto HF diet had impaired glucose tolerance if their dams were on HF diet, experienced prenatal stress, or both.

Conclusions – Our data demonstrate that prenatal stress and/or high fat diet during the intrauterine or postnatal environment affects offspring in a manner that increases their susceptibility to diet-induced obesity and leads to secondary adverse metabolic consequences.
Obesity is a worldwide public health problem and a major contributor to the increased incidence of coronary artery disease, hypertension and type II diabetes (1,2). In addition, and perhaps more disturbing, there is an escalating prevalence of overweight and obesity among infants and children worldwide (3-5). The trend toward greater obesity in the young raises concern because infant or childhood obesity alone significantly increases susceptibility to adult chronic diseases, including cardiovascular disease, hypertension and diabetes (6). Although it is recognized that genetics plays a role in the development of obesity, genetic factors alone cannot account for the tripling in the prevalence of overweight and obesity over the past 3 decades (7).

The intrauterine environment has a significant influence on the health of offspring and exposure to suboptimal in utero conditions can predispose offspring to adult chronic disease. This concept, originally termed the “Barker Hypothesis”, derives from human and animal studies demonstrating that, for example, exposure to limited resources in utero produces offspring that show maladaptive responses to the ample postnatal nutritional environment and will develop obesity and diabetes (8). Poor maternal nutrition during the Dutch famine resulted in increased incidence of obesity in adult males (9). While undernutrition and growth restriction due to famine is not a health issue in modern Western societies, these conditions do occur with hyperemesis gravidarum, high altitude pregnancy, and pregnancy in women with eating disorders. Based upon this, models of low birth weight or intrauterine growth restriction (IUGR) have been studied and are now well-characterized (10,11). Animal models of altered nutritional conditions in dams during pregnancy (e.g. caloric restriction, low protein diet, gestational diabetes) result in low birth weights and eventually lead to adult conditions such as obesity, hypertension, and diabetes reminiscent of observations reported in human epidemiological studies, particularly when exposed to a high energy diet after birth (12). The mismatched prenatal vs. postnatal nutritional environments results in adverse consequences for the offspring as proposed by the “predictive adaptive hypothesis” (13).

Undernutrition and growth restriction are not the only conditions that impact the long-term health of the offspring. Maternal diets have changed such that dietary fat intake among pregnant mothers has increased in the United States (14). Alterations in maternal diet have lead to a two-fold increase in the incidence of maternal overweight and obesity over the last 20 years. Overnutrition or consumption of Western diets that contain a high amount of dietary fat during pregnancy can also result in metabolic syndrome in offspring (15,16).

Another aspect of the maternal environment that may have significant effects on the developing fetus is stress arising from socioeconomic or psychosocial factors. Psychosocial and socioeconomic challenges activate the hypothalamic-pituitary-adrenal (HPA) axis causing hypersecretion of cortisol, and this in turn has been associated with the development of obesity-related conditions including excessive visceral fat deposition, insulin resistance, dyslipidemia, hypertension, and cardiovascular disease in humans (17). Increased glucocorticoid levels and its associated conditions during pregnancy can have significant long-term effects on the developing fetus. Prenatal stress in rodent and primate models has been implicated in altered stress responsivity (18), increased anxiety-like behavior (19), schizophrenia (20,21), cognitive impairments (20) and reduced neurogenesis (22). While exogenous glucocorticoid administration to pregnant rats during gestation has been linked to later
development of hypertension, hyperglycemia and features of the metabolic syndrome (23,24), there are a limited number of studies that have directly examined the consequences of prenatal stress on energy homeostasis (25-27).

The neural pathways that regulate stress responses are also involved in maintaining metabolic homeostasis and the available literature strongly suggests interactions between the two systems (28). In light of the current dietary and stress environment that many humans live in, it is important to address whether prenatal stress, in addition to a high fat diet, may exacerbate the effects of the high fat diet alone. Animal models provide the ability to study the long-term consequences of maternal diet or prenatal stress on offspring while allowing for control over variables that human studies do not afford. We hypothesize that prenatal stress or maternal high fat diet consumption will predispose offspring to metabolic side effects that will be exacerbated by weaning on to high fat diet.

**RESEARCH DESIGN AND METHODS**

**Animals.** Timed-pregnant (2nd parity) female Sprague-Dawley rats (Charles River, Kingston, NY) were received on gestation day 2 (GD 2). Animals were individually housed in conventional tub cages with *ad libitum* access to food and water in an environmentally controlled room maintained on a 12h:12h light:dark cycle with light onset at 0600h. All animal procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Pregnant rats were assigned to two diet groups: standard chow diet (CHOW; Harlan Teklad 7018, 17% kcal from fat; n=21) or high fat diet (HF; Research Diets, D12492, 60% kcal from fat, n=21). All dams remained on their respective diets from GD 2 throughout gestation and lactation. Dams were weighed and food intake was measured daily throughout gestation.

**Variable stress.** Beginning on GD 14, 10 dams from each diet group (n=10, CHOW-STRESS and HF-STRESS) were subjected to an 8-day schedule of variable stress (Table 1)(20). Each stressor was applied during the light cycle unless noted otherwise. We selected a variable stress paradigm for our studies in order to prevent the animals from habituating to the stress as has been documented using predictable stressors such as repeated restraint stress (29). Stress was restricted to the 3rd week of gestation because the neural circuits that regulate the HPA axis and energy homeostasis, including the hypothalamus, cortex and hippocampus, are rapidly developing during this period (30).

The remaining control dams were exposed to normal animal room husbandry practices in the animal facility (n=11, CHOW-CON and HF-CON).

Tail blood samples were collected from all dams on the morning of GDs 14 (pre-stress) and 21 (post-stress) for measurement of basal plasma corticosterone, leptin and insulin.

The day a litter was found was designated postnatal day 0 (PND 0). On PND 1, litters were culled to 10 pups each (5 male and 5 female). Only litters that contained 10 or more pups were included.

**Body weight.** Pups and dams were weighed once weekly beginning on PND 1. Pups were weaned on PND 21 and housed in groups of 2-3 by gender and treatment group. Half of each group (n=4-5 pups) were weaned on to either CHOW or HF diet.

**Glucose tolerance test (GTT).** On PND 22 and PND 70 rats were food deprived overnight for 16 hours with only water available. A baseline blood sample was taken via a small tail nick for determination of plasma insulin. Baseline fasted blood glucose was determined at the same time by a handheld glucometer (Freestyle, TheraSense,
An oral gavage of glucose (2.0 g/kg body weight, 20% glucose in sterile water solution) was then administered. Blood samples were collected at 15, 30, 45, 60, and 120 minutes following glucose gavage to determine plasma insulin levels. Blood glucose was determined at each time point using the glucometer.

**Sacrifice.** Male pups (n=1/litter) were sacrificed at PND 1, 7, 14, 21 and 90 by decapitation. Blood was collected into a heparinized microcentrifuge tube, centrifuged at 4º C to collect plasma and stored at -80º C for hormone analysis. Fat pads (dorsosubcutaneous, inguinal, retroperitoneal) were unilaterally dissected and weighed. The entire subscapular brown adipose tissue (BAT) fat pad was removed and weighed.

**Endocrine assays.** Plasma hormone concentrations were determined by commercially available radioimmunoassay kits for corticosterone (for rats and mice, MP Biomedicals, Solon, OH), leptin and insulin (both for rat from Millipore, Billerica, MA). Inter- and intra-assay variability for each assay were as follows: corticosterone, 6.5-7.1% and 4.4-10.3%; leptin, 3.0-5.7% and 2.0-4.6%; and insulin, 8.5-9.4% and 1.4-4.6%.

**Statistical analysis.** Data were analyzed by Statistica 7.0 (Systat, Tulsa OK) by ANOVA, repeated measures ANOVA, or t-tests for independent samples as appropriate. Subsequent comparisons between groups used Newman-Keuls procedures. Data are presented as mean ± standard error of the mean.

**RESULTS**

**Pregnant dams.** There were no significant differences in maternal body weight among the groups prior to stress exposure (Table 2). However, compared with beginning body weight on GD 2, dams maintained on HF diet gained significantly more weight during the first two weeks of gestation compared to dams fed CHOW diet (P<0.05). The greater weight gain in HF dams may be attributable to significantly higher caloric intake of dams fed HF diet during GD 2-14 (P<0.05). There were no significant differences in plasma corticosterone among the groups prior to stress.

Stress during the last week of gestation (GD 14-21) resulted in less body weight gain in STRESS groups (Table 2). Post-hoc analysis revealed that HF-STRESS dams gained significantly less body weight compared to CHOW-CON. However, body weight on the final day of gestation (GD 21) was not different among the groups. Caloric intake was not affected by stress (GD 15-21). In order to assess the effect of stress and consumption of HF diet, we measured plasma corticosterone, leptin and insulin on GD 21. At this timepoint, plasma corticosterone was significantly higher in the variable stress groups compared to non-stressed controls (P<0.05) indicating that exposure to variable stress resulted in HPA axis activation and increased circulating glucocorticoid levels. There was an overall effect of diet on plasma leptin levels following 20 days of HF diet feeding (P<0.05). Leptin was significantly elevated in the HF-CON group compared to CHOW fed groups. Since leptin is secreted by adipocytes and is highly correlated to adiposity these data suggest that even though they were not significantly heavier, the HF diet dams had greater body adiposity compared to the CHOW fed dams. Blood glucose (data not shown) and plasma insulin were not different among the groups suggesting that high fat feeding did not result in symptoms of gestational diabetes in HF-fed dams.

**Neonatal offspring.** There were no significant differences in litter size (CHOW-CON, 15.3±0.7; CHOW-STRESS, 15.3±0.9; HF-CON, 14.6±0.9; HF-STRESS, 14.5±0.7) or male:female ratios among the groups (CHOW-CON, 0.51±0.05; CHOW-STRESS,
There was an overall effect of maternal STRESS resulting in higher birth weight of both males ($P<0.05$) and females ($P<0.05$). By PND 21, there were significant effects of both maternal DIET and STRESS on body weight of male and female pups (Fig. 1A & 1B). Fat pad (retroperitoneal and subcutaneous pads) weight as a percentage of body weight was reliably higher in both male ($P<0.01$) and female ($P<0.001$) pups from dams that were fed HF diet during gestation and lactation (Fig. 1A & 1B inset). Thus, these data suggest that the increase in body weight on PND 21 in pups from dams on HF diet is attributed, at least in part, to increased adiposity. Subscapular BAT was not different among the groups (data not shown).

Plasma leptin was elevated at birth in male pups from HF-STRESS dams ($P<0.05$; Fig. 2A). By PND 7, maternal HF diet resulted in significantly higher plasma leptin levels compared to pups of CHOW fed dams and this effect persisted throughout the pre-weaning period ($P<0.05$). Pups from HF-STRESS dams had significantly greater blood glucose levels on PND 7 and 14, and by PND 21 both HF-CON and HF-STRESS had elevated blood glucose compared to CHOW-CON ($P<0.05$; Fig. 2B). There was no difference in plasma insulin from PND 1 through 14, however, by PND 21 insulin was significantly greater in pups from HF diet dams (HF-CON, $P<0.01$; HF-STRESS, $P<0.05$; Fig. 2C). Plasma corticosterone was significantly lower on PND 1 in pups from STRESS dams ($P<0.05$) regardless of maternal diet, but there were no differences among the groups for the remainder of the pre-weaning period (Fig. 2D).

At weaning, male and female pups were challenged with a glucose tolerance test (GTT). There were no significant differences between males and females within each group in any of the measures associated with the GTT, therefore, the data have been combined in Fig. 3. Following a 16-hr food deprivation period, there was an overall effect of HF diet in elevating baseline blood glucose (Time 0). While there was no difference in blood glucose at 15 min, it remained elevated in HF-CON and HF-STRESS pups at 30, 45, 60 and 120 min. Glucose area under the curve (AUC) was higher in HF diet pups compared to CHOW pups indicating that pups from dams fed HF diet cleared the glucose load slower than pups from dams on CHOW diet ($P<0.001$; Fig. 3A). There was no significant effect of STRESS on glucose clearance at weaning. There was a main effect of maternal HF diet on insulin secretion in response to the glucose load. At 30, 45, 60 and 120 min, plasma insulin remained higher in both HF-CON ($P<0.05$) and HF-STRESS ($P<0.01$) pups compared to CHOW pups. Insulin AUC was higher in both HF-CON ($P<0.05$) and HF-STRESS ($P<0.01$) pups compared to pups from CHOW dams (Fig. 3B). Together, these data suggest that maternal HF diet resulted in offspring that cleared glucose more slowly and required greater insulin to do so.

**Adult offspring.** On PND 21, half of the male and female offspring were weaned on to CHOW diet and half on to HF diet. When pups were weaned on CHOW diet, there were no longer any significant differences in body weight among the groups in either males or females at PND 70 (Fig. 4A & 4B, open bars). Body composition was not different among males but there was a significant increase in % body fat in HF-STRESS females compared to CHOW-CON (Fig. 4C & 4D, open bars, $P<0.05$). Plasma insulin after an overnight fast was elevated in the HF-STRESS offspring ($P<0.05$), but there were no significant differences among the groups in plasma leptin or fasted blood glucose (Table 3, CHOW WEAN). When adult male offspring (PND 70) were again challenged with a GTT, there were no differences among the groups in glucose clearance (Fig. 5A).
Offspring from HF fed dams had higher fasting insulin and showed a prolonged elevation of insulin at 60 and 120 min although there was no overall effect on insulin AUC (Fig. 5C). However, among females, the HF-STRESS group cleared glucose slower and had greater glucose AUC compared to CHOW-CON and CHOW-STRESS groups (Fig. 6A; \( P<0.05 \)). The CHOW-STRESS group had lower insulin AUC compared to CHOW-CON suggesting that this group may be more efficient in clearing the glucose load (Fig. 6C).

When male and female pups were weaned on to a HF diet on PND 21, those pups that were from dams in the HF diet, STRESS, or the combination HF-STRESS group gained more weight compared to pups from the CHOW-CON dams (Fig. 4A & 4B, black bars). The increase in body weight could be attributed to significantly greater subcutaneous and retroperitoneal fat pad weights (\( P<0.05 \); Fig. 4C & 4D, black bars) and this was associated with elevated plasma leptin levels in CHOW-STRESS and HF-CON groups (\( P<0.05 \); Table 3).

When male offspring were challenged with a GTT at PND 70 after being weaned on to a HF diet, those from dams in the HF diet, STRESS, or the combination HF-STRESS group cleared glucose more slowly relative to male offspring in the same groups weaned on CHOW diet (Fig. 5B; \( P<0.05 \)). Overall, STRESS male offspring required greater insulin to clear the glucose load regardless of maternal diet compared to those pups weaned on CHOW diet (\( P<0.05 \); Fig. 5D).

Among female offspring that were weaned on HF diet, CHOW-CON and CHOW-STRESS groups cleared glucose slower than HF-CON and HF-STRESS groups (\( P<0.05 \); Fig. 6B). Although the offspring weaned on HF diet had greater insulin responses compared to those weaned on CHOW, there was no significant difference among the HF weaned groups in insulin AUC (Fig. 6D).

**DISCUSSION**

The intrauterine environment is critical to fetal development and perturbations of this environment can have significant short- and long-term consequences on the offspring. A growing body of epidemiological data demonstrates that obesity and other metabolic disease may have developmental origins and determination of mechanisms contributing to those conditions may lead to treatment strategies and early interventions to prevent these disorders. We hypothesized that exposure to prenatal stress or maternal high fat diet *in utero* would predispose offspring to develop obesity and that weaning on to a high fat diet would exacerbate this effect. Our data suggest that exposure to maternal prenatal stress or high-fat diet feeding results in offspring that gain more weight and have greater adiposity than controls. Consistent with their body composition, offspring of STRESS or HF diet dams are hyperleptinemic and hyperinsulinemic at weaning. Males and females are similarly affected by maternal prenatal stress and HF diet resulting in impaired glucose tolerance at weaning. While weaning on standard CHOW diet appears to normalize early obesity, the combination of maternal prenatal stress and HF diet resulting in impaired glucose tolerance at weaning. While weaning on standard CHOW diet appears to normalize early obesity, the combination of maternal prenatal stress and HF diet continued to have some effects on glucose tolerance in both male and female offspring. Weaning onto HF diet resulted in obesity and impaired glucose tolerance in offspring exposed to prenatal stress, HF diet, or both. Together the data suggest that maternal prenatal stress or HF diet alters susceptibility of offspring to diet-induced obesity and its metabolic consequences.

Although HF diet dams were not heavier, their plasma leptin levels were elevated on GD 21 suggesting that they had greater adiposity. The offspring that were born to dams on HF diet were heavier at birth,
remained heavier throughout the suckling period, and had impaired glucose tolerance at weaning suggesting that they were developing insulin resistance. Pups of HF diet dams also were hyperleptinemic and hyperinsulinemic by the time they were weaned. Prior studies that have attempted to identify the effects of prolonged maternal high fat diet consumption have been difficult to interpret due to concurrence of high fat diet exposure and maternal obesity (31). Maternal obesity is usually associated with other co-morbid conditions such as gestational diabetes that also has a significant influence on the phenotype of the offspring independent of dietary fat consumption during gestation. Indeed, offspring of rodents with gestational diabetes show increased adiposity, impaired pancreatic function, impaired glucose tolerance, and altered hypothalamic development (32-34). For these reasons, the dams in our study were provided with HF diet only during gestation and lactation. Although dams in the current study were hyperleptinemic indicating that they had greater adiposity after 3 weeks on HF diet, there were no differences in plasma concentrations of insulin or glucose suggesting that they had not developed signs of gestational diabetes. It is unclear from this experiment whether maternal HF diet consumption during gestation or lactation had a greater influence since HF dams remained on HF diet throughout these periods. Future studies with cross-fostered control groups will be required to make this distinction.

Baseline CORT was elevated in dams in the STRESS group suggesting that exposure to variable stress during the 3rd week of gestation was effective. Maternal prenatal stress in our study resulted in higher birth weight in both male and female offspring and this occurred in the absence of significant differences in litter size or male-female ratios. By PND 21, both male and female STRESS offspring had greater body weight and showed signs of impaired glucose tolerance suggesting that prenatal STRESS has early consequences for the offspring. In contrast to the effect of HF diet on endocrine measures, the only hormone that was significantly different in the STRESS offspring was plasma CORT which was lower in the STRESS group compared to CON pups at birth. This may be a compensatory response resulting from the STRESS dams’ elevated plasma CORT at parturition. It is not known whether negative feedback control of corticosterone secretion is operational at this early age.

Prior studies examining the effects of prenatal stress on birth weights have produced mixed results. Some report decreased birth weights resulting from prenatal stress (26,35,36) and others show increased birth weights (27) or no difference compared to controls (25). One possible explanation to account for some of the difference is that those studies reporting lower birth weights used the immobilization stress paradigm that also results in decreased food intake and body weight gain in the dams. Since stress-induced maternal undernutrition and decreased body weight gain during pregnancy has consequences similar to those of intrauterine growth restriction, parceling out the direct effects of stress is difficult in these models. The variable stress schedule that we used did not significantly affect maternal food intake of STRESS dams during the stress period. Epidemiological reports in humans indicate that the birth weight to adult fat mass picture has developed into a “U” shaped curve such that being very light or very heavy at birth may predispose offspring to cardiovascular disease, diabetes, obesity and some types of cancer (15,37). Therefore, while the findings regarding the effects of prenatal stress on birth weight are variable, the different models may operate through different mechanisms depending on the type, intensity, and timing of stress during gestation (38).
Maternal obesity and high fat diet feeding have been associated with hyperphagia and obesity in offspring, particularly when challenged postnataally with a hypercaloric diet (39). We hypothesized that prenatal stress against a background of HF diet feeding would result in an additive or synergistic effect on the offspring. The data at the time points examined suggest that there is no increased effect of the combination of stress and HF diet. An alternative possibility was that maternal HF diet would have a “beneficial” effect in attenuating the stress response of the dam and, in turn, lessen its impact on the developing fetus. Dallman and colleagues have demonstrated that high fat diet can attenuate the HPA axis response to stress (40). We did not note either a detrimental or beneficial effect of maternal HF diet on top of the effects of stress.

In adulthood, offspring exposed to STRESS or HF or both gained significantly more body weight and adiposity, but only if they were weaned onto a HF diet. Consistent with increased body weight and adiposity on HF diet in adulthood, animals from STRESS or HF dams also continued to have impaired glucose tolerance. In contrast, when offspring were weaned on to CHOW, body weight, body composition, and glucose tolerance differences among the groups were no longer as pronounced. It is important to note that the data reported here are from young adult offspring and do not preclude the possibility that metabolic disorders may occur more slowly, emerging later in life.

Overall, our data suggest that prenatal conditions can alter the susceptibility of offspring to future metabolic challenge, in this case HF diet resulting in obesity. This concept has been proposed as the “two-hit” model and suggests that genetic or environmental factors disrupt early development and produce increased susceptibility to disease including Parkinson’s disease (41,42) and schizophrenia (43,44). The “first hit” disruptions that occur during early development set the stage for long-term vulnerability to a “second hit” that occurs later in life and leads to pathology. Thus, it appears that prenatal stress or maternal HF diet is not the direct cause of increased body weight or disturbances in energy homeostasis, but instead increases future susceptibility. Identification of the pathways and mechanisms that produce long-term vulnerability in response to early environmental disruption will facilitate development of clinical intervention and prevention strategies to reduce the incidence of disease. Using these animal models we are now able to extend our studies to determine the potential mechanisms through which prenatal stress or HF diet may program systems that regulate body weight and control food intake. There is evidence that hormones such as corticosterone, insulin and leptin can cross the placental-fetal barrier to potentially affect fetal development (45-47) and, thus, it is likely that fetuses have hormone levels that mirror those of their dams. The significant changes in endocrine parameters in offspring throughout the early postnatal period reported here represent reasonable candidates for “metabolic programming” that occurs in response to HF diet consumption or maternal stress (48). Both leptin and insulin are trophic factors that act during the pre- and postnatal periods and can significantly affect development of neural systems important in the maintenance of energy homeostasis (49). Similarly, stress and excess glucocorticoids may have an effect prenatally by programming increased susceptibility to stress in adulthood. Although placental 11β-hydroxysteroid dehydrogenase (11β-HSD) serves to buffer the developing fetus from excess glucocorticoids in response to acute stress, the upregulation of 11β-HSD is often insufficient during chronic stress (50). Future studies are required to explore the role of 11β-HSD in the phenotypes we observed.
The intrauterine period is a critical time of development and evidence shows that disturbances to the fine balance in utero can have significant and persistent consequences for the offspring. The development of relevant animal models is required to examine the etiology of metabolic disorders and to determine the mechanisms for greater predisposition to those disorders.

ACKNOWLEDGEMENTS
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Prenatal stress and HF diet effect on rat offspring


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Table 1. Schedule of variable stress during gestation.

<table>
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<th>Gestational Day</th>
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<td>14</td>
<td>MORNING</td>
<td>NOON</td>
<td>AFTERNOON</td>
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<tr>
<td>14</td>
<td>Restraint – 60 min</td>
<td>Swim - 15 min at room</td>
<td>Restraint – 60 min</td>
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<tr>
<td>15</td>
<td>Cold exposure (4 °C) – 6 hr</td>
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<td></td>
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<tr>
<td>16</td>
<td>Swim – 15 min</td>
<td>Restraint – 60 min</td>
<td>Swim – 15 min</td>
</tr>
<tr>
<td>17</td>
<td>Swim – 15 min</td>
<td>Swim – 15 min</td>
<td>Lights on during dark</td>
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<td>18</td>
<td>Social stress – group housing – 12 hr</td>
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<tr>
<td>19</td>
<td>Restraint – 60 min</td>
<td>Swim – 15 min</td>
<td>Restraint – 60 min</td>
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<tr>
<td>20</td>
<td></td>
<td>Cold exposure (4 °C) – 6 hr</td>
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<tr>
<td>21</td>
<td>Swim – 15 min</td>
<td>Restraint – 60 min</td>
<td>Swim – 15 min</td>
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Table 2. Maternal body weight, food intake, and endocrine measures during gestation. CORT = corticosterone. * $P < 0.05$ vs. CHOW-CON, † $P < 0.05$ vs. CHOW-CON, CHOW-STRESS.

<table>
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<tr>
<th></th>
<th>CHOW-CON (n = 11)</th>
<th>CHOW-STRESS (n = 10)</th>
<th>HF-CON (n = 11)</th>
<th>HF-STRESS (n = 10)</th>
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<tr>
<td>Body weight, GD 2 (g)</td>
<td>301.4 ± 12.1</td>
<td>308.3 ± 20.2</td>
<td>300.0 ± 14.2</td>
<td>316.9 ± 16.9</td>
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<td>Body weight, GD 14 (g)</td>
<td>386.0 ± 14.9</td>
<td>401.9 ± 17.1</td>
<td>408.4 ± 17.3</td>
<td>424.7 ± 19.7</td>
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<td>Body weight gain (g)</td>
<td>84.7 ± 5.0</td>
<td>93.6 ± 7.3</td>
<td>108.4 ± 7.3*</td>
<td>107.8 ± 4.7*</td>
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<td>Food intake (kcal/day)</td>
<td>79.6 ± 4.7</td>
<td>86.2 ± 4.5</td>
<td>109.3 ± 6.6†</td>
<td>111.2 ± 5.8†</td>
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<td>CORT, GD 14 (ng/ml)</td>
<td>100.5 ± 13.9</td>
<td>126.0 ± 33.0</td>
<td>91.1 ± 20.0</td>
<td>125.4 ± 7.5</td>
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<td><strong>Gestation day 15-21 (stress period)</strong></td>
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<tr>
<td>Body weight, GD 21 (g)</td>
<td>474.9 ± 18.3</td>
<td>475.1 ± 21.4</td>
<td>488.8 ± 21.1</td>
<td>488.6 ± 21.1</td>
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<td>Body weight gain (g)</td>
<td>88.9 ± 4.1</td>
<td>73.2 ± 8.2</td>
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<td>Food intake (kcal/day)</td>
<td>82.5 ± 3.2</td>
<td>85.0 ± 4.0</td>
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<td>CORT, GD 21 (ng/ml)</td>
<td>186.2 ± 31.8</td>
<td>389.6 ± 78.0*</td>
<td>269.8 ± 74.2</td>
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<td>Leptin, GD 21 (ng/ml)</td>
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<td>Insulin, GD 21 (ng/ml)</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>
Table 3. Endocrine measures in adult male offspring. *$P < 0.05$ vs. CHOW-CON, †$P < 0.05$ vs. corresponding CHOW WEAN.

<table>
<thead>
<tr>
<th></th>
<th>CHOW-CON (n = 6)</th>
<th>CHOW-STRESS (n = 4)</th>
<th>HF-CON (n = 4)</th>
<th>HF-STRESS (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>14.5 ± 2.5</td>
<td>14.4 ± 3.8</td>
<td>17.6 ± 3.7</td>
<td>18.2 ± 3.3</td>
</tr>
<tr>
<td>Fasted glucose (mg/dl)</td>
<td>69.3 ± 5.5</td>
<td>72.8 ± 3.9</td>
<td>71.0 ± 4.3</td>
<td>75.0 ± 2.8</td>
</tr>
<tr>
<td>Fasted insulin (ng/ml)</td>
<td>1.01 ± 0.17</td>
<td>1.13 ± 0.20</td>
<td>1.48 ± 0.25</td>
<td>1.98 ± 0.42 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CHOW-CON (n = 3)</th>
<th>CHOW-STRESS (n = 4)</th>
<th>HF-CON (n = 5)</th>
<th>HF-STRESS (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>16.4 ± 5.8</td>
<td>25.8 ± 3.2 †</td>
<td>30.7 ± 4.6 *†</td>
<td>23.1 ± 3.6</td>
</tr>
<tr>
<td>Fasted glucose (mg/dl)</td>
<td>85.5 ± 9.1 †</td>
<td>87.3 ± 5.4 †</td>
<td>95.8 ± 7.9 †</td>
<td>91.1 ± 5.3 †</td>
</tr>
<tr>
<td>Fasted insulin (ng/ml)</td>
<td>1.25 ± 0.39</td>
<td>2.43 ± 0.37 * †</td>
<td>1.68 ± 0.30</td>
<td>1.99 ± 0.31</td>
</tr>
</tbody>
</table>
Figure 1A & 1B. Body weight and fat as a percentage of body weight (inset graphs) of offspring through PND 21. A: Male offspring in each litter were weighed on postnatal days (PND) 1, 7, 14, and 21. B: Female offspring in each litter were weighed on PND 1, 7, 14, and 21. Fat as a percentage of body weight was determined on PND 21 (inset graphs). CHOW-CON (n = 11 litters), CHOW-STRESS (n = 10 litters), HF-CON (n = 11 litters), HF-STRESS (n = 10 litters), C-C = CHOW-CON, C-S = CHOW-STRESS, H-C = HF-CON, H-S = HF-STRESS. * main effect of STRESS, $P < 0.05$ vs. CON; † main effect of DIET, $P < 0.05$ vs. CHOW; ‡ main effect of DIET and STRESS, $P < 0.05$. 

A. 

B.
Figure 2A - 2D. Endocrine parameters in male offspring PND 1-21. A: Plasma leptin. B: Blood glucose. C: Plasma insulin. D: Plasma corticosterone. CHOW-CON (n = 11), CHOW-STRESS (n = 10), HF-CON (n = 11), HF-STRESS (n = 10). * P < 0.05 vs. CHOW-CON, CHOW-STRESS, HF-CON; † P < 0.05 vs. CHOW-CON, CHOW-STRESS, ‡ P < 0.05 vs. CHOW-CON.
Figure 3A & 3B. Glucose tolerance test (2.0 g/kg, oral gavage) for male and female pups on PND 23. A: Blood glucose following oral administration of glucose. B: Plasma insulin following oral administration of glucose. The integrated area under the curve (AUC) was determined for glucose and insulin using the trapezoidal method. CHOW-CON (n = 8), CHOW-STRESS (n = 8), HF-CON (n = 8), HF-STRESS (n = 8). C-C = CHOW-CON, C-S = CHOW-STRESS, H-C = HF-CON, H-S = HF-STRESS. * P < 0.05 CHOW-STRESS, HF-CON, HF-STRESS vs. CHOW-CON; † P < 0.05 HF-CON, HF-STRESS vs. CHOW-CON, CHOW-CON, CHOW-STRESS.
**Figure 4A - 4D.** Body weight and fat as a percentage of body weight for adult male (A) and female (B) offspring. Fat as a percentage of body weight for males (C) and females (D) is expressed as weight of dorsosubcutaneous, inguinal, retroperitoneal fat pads as a percentage of body weight. Males weaned on CHOW: CHOW-CON (n = 6), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Males weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4); Females weaned on CHOW: CHOW-CON (n = 5), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Females weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4). *P < 0.05 vs. CHOW WEAN; †P < 0.05 vs. CHOW-CON.
**Figure 5A – 5D.** Glucose tolerance test in males on PND 70. Blood glucose (A & B) and plasma insulin (C & D) was determined for 2 hours following oral administration of glucose for male offspring weaned on to CHOW (A & C) or HF diet (B & D). Males weaned on CHOW: CHOW-CON (n = 6), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Males weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4). * DIET main effect, \( P < 0.05 \); † STRESS main effect, \( P < 0.05 \); ‡ DIET X STRESS interaction, \( P < 0.05 \).

**Figure 5A – 5D Insets:** The integrated area under the curve (AUC) was determined for glucose and insulin using the trapezoidal method. C-C = CHOW-CON, C-S = CHOW-STRESS, H-C = HF-CON, H-S = HF-STRESS. * \( P < 0.05 \) vs. corresponding CHOW WEAN group; † \( P < 0.05 \) vs. CHOW-CON, CHOW-STRESS; ‡ \( P < 0.05 \) vs. CHOW-CON, HF-CON.
Figure 6A – 6D. Glucose tolerance test in females on PND 70. Blood glucose (A & B) and plasma insulin (C & D) was determined for 2 hours following oral administration of glucose for female offspring weaned on to CHOW (A & C) or HF diet (B & D). Females weaned on CHOW: CHOW-CON (n = 5), CHOW-STRESS (n = 4), HF-CON (n = 4), HF-STRESS (n = 4); Females weaned on HF: CHOW-CON (n = 4), CHOW-STRESS (n = 4), HF-CON (n = 5), HF-STRESS (n = 4). * DIET main effect, \( P < 0.05 \); † STRESS main effect, \( P < 0.05 \).

Figure 6A – 6D Insets: The integrated area under the curve (AUC) was determined for glucose and insulin using the trapezoidal method. C-C = CHOW-CON, C-S = CHOW-STRESS, H-C = HF-CON, H-S = HF-STRESS. * \( P < 0.05 \) vs. corresponding CHOW WEAN group; † \( P < 0.05 \) vs. CHOW-CON, CHOW-STRESS; ‡ \( P < 0.05 \) vs. CHOW-CON, HF-CON.