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Rat Maternal Obesity and High Fat Diet Program Offspring Metabolic Syndrome

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Abstract

Objective—We determined the potential programming effects of maternal obesity and high fat (HF) diet during pregnancy and/or lactation on offspring metabolic syndrome.

Study Design—A rat model of maternal obesity was created using a HF diet prior to and throughout pregnancy and lactation. At birth, pups were cross fostered thereby generating four paradigms of maternal diets during pregnancy/lactation: (1) control (Con) diet during pregnancy and lactation - Con/Con, (2), HF during pregnancy and lactation - HF/HF, (3) HF during pregnancy alone - HF/Con, and (4) HF during lactation alone - Con/HF.

Results—Maternal phenotype during pregnancy and the end of lactation evidenced markedly elevated body fat and plasma corticosterone levels in HF dams. In the offspring, maternal HF diet during pregnancy alone programmed increased offspring adiposity, though with normal body weight, whereas maternal HF diet during lactation increased both body weight and adiposity. Metabolic disturbances, particularly that of hyperglycemia, were apparent in all groups exposed to maternal HF diet (during pregnancy and/or lactation), though differences were apparent in the manifestation of insulin resistant versus insulin deficient phenotypes. Elevated systolic blood pressure was manifest in all groups implying that exposure to an obese/HF environment is disadvantageous for offspring health, regardless of pregnancy or lactation periods. Nonetheless,

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the underlying mechanism may differ as offspring that experienced *in utero* HF exposure had increased corticosterone levels.

Conclusion—Maternal obesity/HF diet markedly impact offspring body composition and the risk of metabolic syndrome dependent upon period of exposure during pregnancy and/or lactation.

Keywords

pregnancy; lactation; rat; obesity; impaired glucose tolerance; hypertension

INTRODUCTION

An epidemic of metabolic syndrome is well recognized within the United States. Currently, 65% of adult Americans are overweight and 30% obese, though a marked increase in obesity is apparent from childhood through adolescence.[1] Notably, pre-pregnancy obesity prevalence continues to increase as well.[2,3] Among women presenting for prenatal care, the incidence of obesity has doubled since 1980.[4] Not only do women begin pregnancy at a higher body mass index, but women also gain excess gestational weight.[5] Thus, clinicians caring for pregnant women are increasingly caring for women who are overweight or obese.

It is well established in human studies and animal models that maternal nutritional factors during pregnancy may have marked effects on fetal growth and ultimately influence the offspring's predisposition to obesity.[6,7] Studies which have examined the developmental origins of adult obesity have demonstrated that maternal obesity, weight gain during pregnancy, and/or the presence of gestational diabetes are each associated with an increased risk of the offspring becoming obese during childhood and/or as adults. Recent human studies have demonstrated that among these factors, maternal pre-pregnancy weight may be the most predictive of offspring obesity.[8,9] Animal studies have begun to examine the mechanisms of fetal programming, with evidence that maternal obesity and a western HF diet program fetal adipose tissue to promote increased adipogenesis and hypothalamic neural pathways to promote appetite as compared to satiety.[10–12]

Breastfeeding has been strongly encouraged for postpartum women in the United States, with evidence suggesting significant benefits in newborn immune function, nutrition, maternal weight loss, and maternal-newborn bonding.[13,14] However, it is recognized that maternal breast milk reflects in part, the maternal diet, and maternal obesity and the HF western diet may contribute to higher fat content in breast milk.[15] Thus, maternal obesity may affect breast milk-induced newborn programming, independent of maternal obesity effects on fetal programming.

In view of the potential programming effects of maternal obesity during both pregnancy and lactation, we sought to examine the effects of each of these periods on programmed metabolic syndrome in rats. We hypothesized that offspring exposed to maternal obesity during both pregnancy and lactation would be more obese and exhibit a greater degree of metabolic abnormalities.

MATERIAL AND METHODS

Maternal Diet and Studies

A rat model of maternal obesity was created using HF diet diet prior to and through pregnancy and lactation was utilized. Studies were approved by the Animal Research Committee of Harbor-UCLA Medical Research and Education Institute and were in accordance with the American Association for Accreditation of Laboratory Care and National Institutes of Health guidelines. Sprague Dawley rats (Charles River Laboratories, Inc, Hollister, CA) were housed in a facility with constant temperature and humidity and on a controlled 12 h light-12 h dark cycle. Beginning as weanlings, female rats were fed a HF (60% k/cal fat, Purified Diet58Y1, New Brunswick, NJ; N=16) or Control (Con; 10% k/cal fat, Purified Diet 58Y2, New Brunswick, NJ; N=16) diet. The nutrient composition is given in Table 1. At 11 weeks of age, rats were mated and continued on their respective diets during pregnancy and lactation.

Maternal body weights and their food intake were recorded daily. In addition, maternal blood was obtained via tail bleed at term (gestational age e20) and at end of lactation (21 days post-partum) for glucose, lipid and hormonal analysis (details below). The food was removed 1 hour prior to blood sampling. Further, at end of lactation, dams underwent a non-invasive dual-energy x-ray absorptiometry (DEXA) scan (details below) for evaluating percentage body fat and lean body mass.

The Offspring

At birth, pups were culled to eight per litter (4 males and 4 females) to normalize rearing, and were cross-fostered, thereby generating four paradigms of maternal diets during pregnancy/lactation: (1) Con diet during pregnancy and lactation - Con/Con, (2), HF during pregnancy and lactation - HF/HF, (3) HF during pregnancy alone - HF/Con, and (4) HF during lactation alone - Con/HF. At 3 week of age, offspring in each of the four groups were housed individually and 2 male and 2 female per litter were weaned to normal fat diet (10% k/cal).

Body Weights and Food Intake—Each litter from the four groups was weighed weekly, and the weight of an individual pup was calculated from it (i.e., litter weight/number of pups). The first weight was recorded at 1 d of age and subsequent weights were taken at 7, 14 and 21 days of age. Thereafter, the body weight and food intake were monitored weekly on individual basis.

Body composition—At 3 and 24 weeks of age, offspring of both sexes underwent non-invasive DEXA scanning using the DXA system with software program for small animal (QDR 4500A, Hologic, Bedford, MA, USA). An in vivo scan of whole body composition was obtained, including lean and fat tissue mass, total mass and percent body fat measurements.

Blood Pressure—Blood pressure was determined in conscious 8 week old male and female offspring using a non-invasive Tail-cuff sphygmomanometry (ML125 NIPB System,

AD Instruments) method. Several cuff sizes were used depending on the weight of the animal. To circumvent the potential problem of restrain-induced stress, the animals were acclimatized for at least one week with placement in the restraint.

Glucose Tolerance Test—At 6 and 24 weeks of age, offspring of both sexes underwent glucose tolerance test (GTT) as follows: After an overnight fast, D-glucose (1mg/g body weight) was injected intraperitoneally in conscious rats. Blood was taken via tail bleed prior to (time 0) and 15, 30, 60, 120 and 180 min after glucose injection.

Blood Collection—One day after birth (~24hrs after birth), the excess pups (4 pups per litter) from Con (n=16) and HF (n=16) groups were decapitated and blood was pooled from pups from the same litter. At ages 3 and 24 weeks, one male and one female from each litter (N=8 per group) were fasted overnight and blood was collected via cardiac puncture in heparinized tubes for plasma analysis.

Plasma Analysis—Plasma insulin, leptin and coritcosterone levels were measured using rat specific commercial radioimmunoassay kits (insulin and leptin RIA kit, LINCO Research Inc., St. Charles, MO; coritcosterone RIA Kit, Diagnostic Systems Laboratories, Inc, Webster, TX). Plasma lipid levels were measured using reagents from Raichem, Inc. (San Diego, CA) and run on an automated Cobas-Mira Chemistry Analyzer (Roche Diagnostic Systems Inc., Sommerville, NJ). Plasma triglycerides (Cat No. 80008) and cholesterol (Cat No. 80015) concentrations were analyzed using Raichem Enzymatic Reagents (with control serum level 1 #83082 and control serum level 2 #83083). Blood glucose determined using Hemocue B-Glucose Analyzer (HemoCue Inc, Mission Viejo, CA).

Statistical Analysis

For all offspring studies at 3, 6, 8 and 24 weeks of age, 8 males and 8 females from 8 litters were studied per group. Differences between Con and the experimental groups were compared using either unpaired t-test (1 day old neonate), repeated measures of ANOVA (body weight and food intake), or ANOVA with Dunnett's post hoc tests (body composition and plasma hormones/metabolites). At ages 1 day and 3 weeks, combined data for males and females are shown since no sex differences were evident. However, at the age 24 weeks, sex differences justified analyzing the data according to sex. Values are expressed as means ± SE.

RESULTS

Maternal Dams

Pregnancy—Maternal body weight was increased at the initiation of pregnancy as per the experimental model. Both HF and Con dams gained nearly identical amounts of weight during the pregnancy (Fig 1A). However, there were marked differences immediately following delivery, as Con dams experienced a significantly greater weight loss from pregnancy day e20 to postnatal day one (100 ± vs. 40g; Fig 1B). Among the four lactation groups, three of the groups demonstrated similar maternal weight change during lactation (Con/Con, Con/HF, HF/HF) with a slight increase in maternal weight through day p10–12

and a slight decrease in maternal weight at the completion of lactation (day p20). The HF dams nursing Con pups evidenced a continued decrease in maternal weight throughout the lactation period.

When assessed by percent of carbohydrate, fat, and protein intake, HF dams consumed a greater percentage of kilocalories via fat and a lower percentage by carbohydrate during both pregnancy and lactation, with nearly identical levels of protein intake as Con dams (Fig 2). The total food intake was similar among HF and Con dams during pregnancy. However, food intake was significantly greater during the terminal portion of lactation among HF dams nursing HF pups, and HF dams nursing Con pups (Fig 3).

In conjunction with maternal obesity and a HF maternal diet, the plasma profile of pregnant dams at gestation day e21 included increased plasma cholesterol and coritcosterone, though HF and Con dams hadsimilar plasma leptin, plasma triglyceride, and blood glucose levels (Table2).

Lactation—At the completion of lactation, there were marked differences among the dams dependent upon the offspring being nursed (Table 3). HF/HF dams demonstrated markedly increased percent body fat and reduced lean body mass as compared to all groups. Notably, the HF/Con dams' body fat was significantly less than the HF/HF, consistent with the loss of maternal body weight among HF/Con dams during lactation. Plasma leptin levels reflected the percent body fat with elevated levels in the HF/HF group. Plasma cholesterol levels, which were elevated among HF dams at the completion of pregnancy, demonstrated a reduction in HF/HF dams as well as HF/Con dams, coinciding with lower levels of plasma triglycerides. Blood glucose levels were similar among all groups, though HF/HF and HF/Con dams demonstrated elevated levels of plasma insulin. Notably, Con/HF dams had markedly elevated levels of plasma corticosterone at the completion of lactation.

The Offspring

Growth—Both Con and HF newborns at day p1 had similar body weights (7.3 ± 0.1; 7.4 ± 0.2, respectively). During the nursing period, offspring weight gain diverged into a rapid weight gain group among HF/HF and Con/HF offspring, that was evident in both males and females by three weeks of age (Fig 4). Females continued the divergent pattern through 30 weeks of age, whereas males exhibited three growth patterns, as follows: HF/HF offspring demonstrated a continued acceleration of body weight gain through 30 weeks of age, Con/HF males demonstrated intermediate growth and HF/Con demonstrating weight gain similar to Con/Con.

Food Intake—Unlike the females, the food intake of male offspring paralleled weight gain, with HF/HF offspring demonstrating the greatest food intake from the end of lactation through 24 weeks of age. Con/HF offspring demonstrated intermediate food intake, slightly greater than Con offspring. Notably, HF/Con offspring demonstrated accelerated food intake through 18 weeks of age, after which food intake normalized to levels of the Con/Con offspring (Fig 5).

Body Composition—Consistent with the weight gain, both HF/HF and Con/HF offspring demonstrated significantly increased percent body fat and a reduction in lean body mass at three weeks of age (Fig 6A). At 24 weeks, all three groups exposed to HF diet during pregnancy and/or lactation demonstrated markedly increased percent body fat as compared to Con/Con (Fig 6B). However, as described previously, only the HF/HF and Con/HF demonstrated significantly increased body mass. HF/Con males and females had significantly reduced lean body mass as compared to respective Con/Con offspring, as measured by both grams and percentage body weight, whereas all three groups exposed to HF diet during pregnancy and/or lactation demonstrated a reduced percentage lean body mass at 24 weeks.

Plasma Profile—At 1 day of age, HF newborns had lower plasma triglyceride, cholesterol, leptin, and corticosterone levels, though similar plasma insulin levels as Con/Con (Table 4).

At three weeks of age, plasma triglyceride levels were significantly increased among HF/HF and Con/HF offspring though there was no difference in plasma cholesterol levels. However, HF/HF and HF/Conl offspring both demonstrated significantly increased plasma leptin, insulin and corticosterone levels as compared to Con/Con and Con/HF. Consistent with the plasma insulin levels, HF/HF and HF/Con offspring demonstrated elevated blood glucose levels (Fig 7A).

At 24 weeks of age, plasma triglyceride levels were increased among the three groups of HF exposure, though only Con/HF males demonstrated increased cholesterol levels. Furthermore, all three groups of HF exposure showed increased levels of glucose, leptin and insulin levels in both genders. However, plasma coritcosterone levels were significantly elevated in HF/HF and HF/Con male and female offspring (Fig 7B).

Glucose Tolerance Test—At 6 weeks of age, glucose tolerance tests were performed in all offspring. HF/Conmales and females demonstrated the greatest area under the curve as compared to the other three groups (Fig 8A). When the glucose tolerance test was repeated at 24 weeks of age, HF/Con offspring again demonstrated the greatest areas under the curve, with intermediate values evident among HF/HF and Con/HF as compared to Con/Con.

Blood Pressure—Among 8 week old male and female offspring, elevated systolic blood pressures were demonstrated in males and females of all three groups exposed to the HF diet during pregnancy and/or lactation (HF/HF, HF/Con, Con/HF) as compared to Con/Con (Fig 9). We were unable to measure blood pressure at 24 weeks of age due to unavailability of tail-cuff size in that range.

Male/Female Comparison—In general, 24 week old male offspring had significantly higher percentage lean body mass, blood pressure, and plasma leptin, insulin and triglyceride levels as compared with female offspring. In contrast, percentage body fat and plasma corticosterone levels were significantly higher in females than males.

DISCUSSION

The results of the present study demonstrate a marked impact of maternal obesity/HF diet on offspring body composition and the risk of metabolic syndrome. Importantly, differential effects on offspring phenotype were observed dependent upon whether exposure to maternal obesity occurred during pregnancy, during lactation, or both the pregnancy and lactation periods.

Although HF dams were significantly heavier at conception and throughout pregnancy, both HF and Con dams gained similar amounts of weight during pregnancy and demonstrated similar total caloric intakes. Recent guidelines for gestational weight gain have advocated lower weight gain among overweight or obese patients as opposed to normal weight women,[16,17] with some studies advocating a zero weight gain goal among morbidly obese patients.[18] Extrapolation from the present rodent studies to humans would suggest that despite a ~33% increment in pre-pregnancy body weight, there is an intrinsic programmed increment in pregnancy weight gain, due in part to fetal mass, maternal fat deposition, and maternal physiologic alterations (e.g., blood volume expansion). Thus, attempts to limit maternal body weight gain may require active intervention strategies. Notably Con dams lost more weight at delivery than HF dams, though the explanation for this is unclear. As litter size and pup weight were similar between the two groups, this weight loss is not a reflection of fetal mass. Alternatively, changes in maternal physiologic adaptations (e.g., maternal blood volume alterations) or in the amniotic/allantois fluid compartments may contribute to the increased maternal weight loss in Con dams.

Con dams nursing either Con or HF pups demonstrated a gradual increase in body weight through the first half of lactation and a decrease in body weight during the last third of lactation, likely associated with the increased pup milk ingestion and pup weight gain. Similar findings were observed in the HF dams nursing HF pups. Surprisingly, the HF dams nursing Con pups demonstrated continued weight loss throughout the lactation period. Nevertheless, both HF dams nursing Con or HF pups demonstrated a marked increase in caloric intake in the last third of lactation. This caloric intake likely served to support the increased weight gain demonstrated by both HF/HF pups and the Con/HF pups. As clarified in the methods section, due to the process we utilized for identifying dams and pups, Con/HF pups were born to Con dams and nursed by HF dams. These findings indicate that maternal caloric intake, and thus, maintenance of maternal body weight is dependent, at least in part, upon the offspring weight gain during lactation. The mechanism for the loss of maternal body weight in the HF dams nursing Con pups is unclear, as the weight gain of these pups was identical to that of the HF/HF pups and the caloric intake of the dams were also identical. Nevertheless, the increased weight loss of HF dams nursing Con pups during lactation was reflected in their body composition and plasma profile, as these dams demonstrated significantly reduced percent body fat, plasma leptin, and plasma insulin as compared to HF dams nursing HF pups at day 21 of lactation.

Consistent with human pregnancy, both Con and HF dams demonstrated elevated plasma cholesterol and triglyceride levels as compared to non-pregnant animals[19–21] and compared to end of lactation values. Despite the HF diet throughout pregnancy and lactation, both HF

dams nursing HF and Con pups demonstrated lower plasma triglyceride and cholesterol levels as compared to Con dams nursing Con or HF pups. Thus, the HF dams transitioned from significantly elevated plasma cholesterol and triglyceride at day 20 of pregnancy to significantly lower values of both analytes at the completion of lactation, despite identical diets during both periods. Thus, it is not dietary changes, but rather metabolism and perhaps gastrointestinal absorption that contribute to these basal levels of cholesterol and triglyceride. It is known that lipid metabolism differs between lean and obese pregnant women with obese women demonstrating an earlier shift from ananabolic to a catabolic state and a predominance of lipolysis as compared with lean women.[22,23] Although maternal hypercholesterolemia and hypertriglyceridemia during pregnancy is believed to contribute to the long term risk of atherosclerosis or hypertension,[24,25] elevated cholesterol and fatty acid profiles during what may be years of lactation may also have an associated risk. Thus, the reduced levels of triglyceride and cholesterol in overweight and obese HF dams during lactation deserves further study to explore mechanisms regulating cholesterol and fatty acid profiles.

Notably, plasma corticosterone levels were markedly elevated in HF dams at term indicating that the effects of diet and/or maternal obesity represented a significant stress factor. HF dams continued to have elevated plasma corticosterone levels at day 21 of lactation, regardless of the pup identification. However, the highest levels of plasma corticosterone were observed in Con dams nursing HF pups, perhaps suggesting that the increased weight gain of these pups (HF/Con) induces a significant maternal stress. Previous studies on rat maternal obesity have made similar observation of elevated maternal and lower offspring coritcosterone levels.[26]

Examination of offspring weight gain demonstrates programming effects of maternal diet during both pregnancy and lactation. Consumption of a HF diet during pregnancy did not impact birth weight and, if limited to the pregnancy, did not impact on the body weights of either males or females as 24 week adults. Consumption of a HF diet during lactation only was associated with increased body weight of male and female offspring at 24 weeks, suggesting that HF diet does not impair milk production. However, consumption of a HF diet during both pregnancy and lactation (HF/HF) resulted in a further increase of body weight of males, though not females, as compared to respective Con/HF at 24 weeks. Thus, the pregnancy HF diet potentiates offspring growth when offspring are also exposed to dams consuming a HF diet during nursing. Offspring food intake appears to be a major contributor to body weight changes in males, as food intake paralleled changes in body weight. Although female food intake showed a similar trend, the lack of statistical difference in food intake despite significant differences in body weight to 24 weeks may suggest that either metabolic efficiency and/or energy expenditure may be programmed by maternal dietary changes during pregnancy and/or lactation. Notably, studies on rodents and sheep offspring suggested a programming effect of maternal diet on energy expenditure in adult offspring.[27–29]

The increased body weight of HF/HF and Con/HF offspring at 24 weeks was associated with increased percent body fat of both males and females. This was accompanied by reduced absolute and percentage lean body mass in Con/HF males and females. These

findings are similar to that described in humans, particularly in the Indian subcontinent, of the “thin-fat” phenotype in which individuals have a normal body weight, but markedly increased body fat and reduced lean body mass.[30] Importantly, it was subscapular rather than abdominal visceral fat that was the preserved, suggesting increased subcutaneous adipose tissue. Similarly, study by Zambrano et al (2010) showed increased subcutaneous fat in offspring exposed to maternal obesity during the fetal and nursing periods. However in the present study, it remains to be determined whether there is a differential effect on visceral versus subcutaneous adipose tissue as a result of HF diet exposure during pregnancy and/or lactation.[31] Notably, all three groups exposed to HF diet during any portion of pregnancy or lactation demonstrated a significant decrease in percent lean body mass as compared to Con offspring.

Despite their normal birth weight, HF pups had lower plasma triglyceride, cholesterol, leptin and coritcosterone levels. Moreover, these values contrasted with elevated maternal levels. Although the lower HF newborn plasma levels are consistent with previous studies[30,31], the mechanism remains unclear and may involve placental effects. Unlike the newborn, the plasma profile of three week old offspring reflected the maternal diet during lactation with HF/HF and Con/HF offspring demonstrating elevated triglyceride levels, and increased leptin. However, at 24 weeks, all groups exposed to HF during pregnancy, lactation, or both, demonstrated elevated plasma triglyceride and plasma leptin levels. Thus, HF during pregnancy alone results in adult, though not weanling, hypertriglyceridemia. Plasma corticosterone levels were elevated in three week old HF/HF and HF/Con offspring and these effects persisted at 24 weeks, again indicating the programming effects of HF diet during pregnancy on offspring endogenous glucocorticoid expression. Similarly, the pregnancy HF diet resulted in elevated blood glucose and plasma insulin levels at three weeks of age (HF/HF HF/Con), although all three HF exposed groups demonstrated elevated blood glucose and plasma insulin at 24 weeks. Despite the elevated glucose and insulin levels in all three groups of HF exposed offspring, only the HF/Con offspring demonstrated an elevated area under the glucose tolerance test at six weeks of age. Nevertheless, these results indicate that the augmented insulin secretion in HF/Con is not adequate for the maintenance of normoglycemia in this group. Consistent with elevated blood glucose and plasma insulin, impaired glucose tolerance with elevated area under the curve in all three HF offspring groups is evident at 24 weeks of age. The markedly elevated plasma insulin levels in HF/HF offspring at 24 weeks is nearly two-fold that of HF/Con and Con/HF. These results suggest that the latter two groups may have inadequate insulin secretion contributing to the plasma hyperglycemia evident at 24 weeks, while the HF/HF offspring have marked hyperglycemia and hyperinsulinemia, indicative of insulin resistant diabetes. Similar characteristics of increased adiposity and insulin resistance have been reported in adult offspring exposed to maternal HF diet prior to mating and throughout pregnancy and lactation.[31]

All groups of male and female HF-exposed offspring demonstrated elevated systolic pressure at eight weeks of age. As noted previously, plasma corticosterone levels were elevated in HF/HF and HF/Conoffspring suggesting that elevated glucocorticoids may contribute to the mechanism of hypertension in this group.[34] However, the mechanism of hypertension in Con/HF offspring likely involves mechanisms other than glucocorticoid

mediated processes. As vasculogenesis continues during the nursing period of maternal HF exposure, it is possible that alterations in vascular and/or cardiac development may alter blood pressure regulation.[35–37]

The overall gender differences, namely that the males exhibit higher lean body mass, blood pressure and plasma triglyceride levels than females, is consistent with prior human and rat data.[38–39] The disparity in gender response to maternal obesity/HF diet was evident only in the offspring exposed exclusively during the nursing period (Con/HF). This was apparent only in the cholesterol levels with the males exhibiting higher levels. Although the cause for sex-dependent response is unknown, previous studies have noted differential impact of early overnutrition on male versus females.[40]

The primary mechanism linking fetal/postnatal growth with glucose impairment and dyslipidemia remains to be determined though studies have implicated alterations in epigenomics, gene expression and signaling factors, including alteration in the milk composition.[41–43] The mechanism of programming resulting from in utero overnutrition likely involves an interacting effects of preexisting maternal obesity and HF diet effects.

In summary, maternal phenotype during pregnancy and the end of lactation evidenced markedly elevated body fat and plasma corticosterone levels in HF dams. The results of the present study demonstrate that maternal HF diet during lactation promotes offspring obesity, and HF diet during pregnancy and lactation results in a further increase in offspring body weight and percent fat mass. HF diet during pregnancy alone programmed increased offspring adiposity (percent body fat), though with normal body weight, simulating the thin/fat human phenotype. Metabolic disturbances, particularly that of hyperglycemia were apparent in all groups exposed to maternal HF diet (during pregnancy and/or lactation), though differences were apparent in the manifestation of insulin resistant vs. insulin deficient phenotypes. Elevated systolic blood pressure was manifest in all groups implying that exposure to an obese/HF environment is disadvantageous for offspring health, regardless of pregnancy or lactation periods. Nonetheless, the underlying mechanism may differ as offspring that experienced in utero HF exposure had increased corticosterone levels. Thus, for both fetal and maternal well-being, pre-pregnancy obesity and western HF diet may have significant adverse impact on long term health. The exposure to HF diet during both pregnancy and lactation results in an altered phenotype and exaggerated obesity when compared to pregnancy alone. However, pregnancy exposure alone also has significant adverse effects, indicating that suggestions to limit nursing or advocate formula feeding to infants of obese/HF mothers would likely not remedy the programming effects of the pregnancy environment. Rather, preconceptual weight loss is optimal to promote the beneficial effects of a pregnancy environment.

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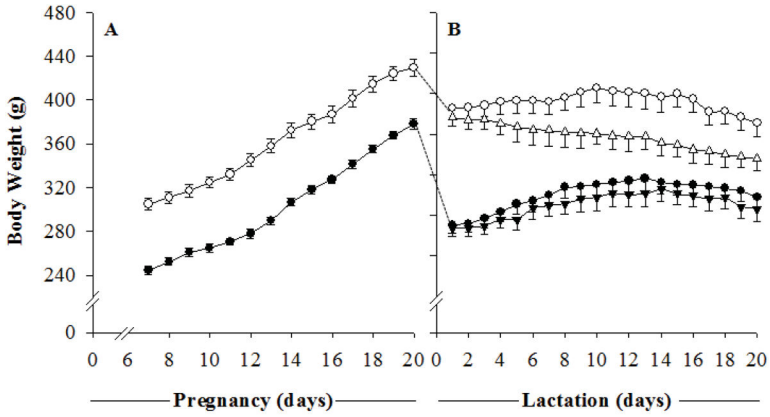

Figure 1. Maternal Body Weights during Pregnancy and Lactation

(A) Daily maternal body weight during pregnancy (e8–e20) of Con (●) and HF dams (○). (B) Daily maternal body weight during lactation (day 2–20) of Con dams nursing Con pups (●), high fat dams nursing high fat pups (○), Con dams nursing high fat pups (▲) and high fat dams nursing Con pups (Δ). Values are mean±SE of N=16 dams per group during pregnancy and N=8 per group during lactation.

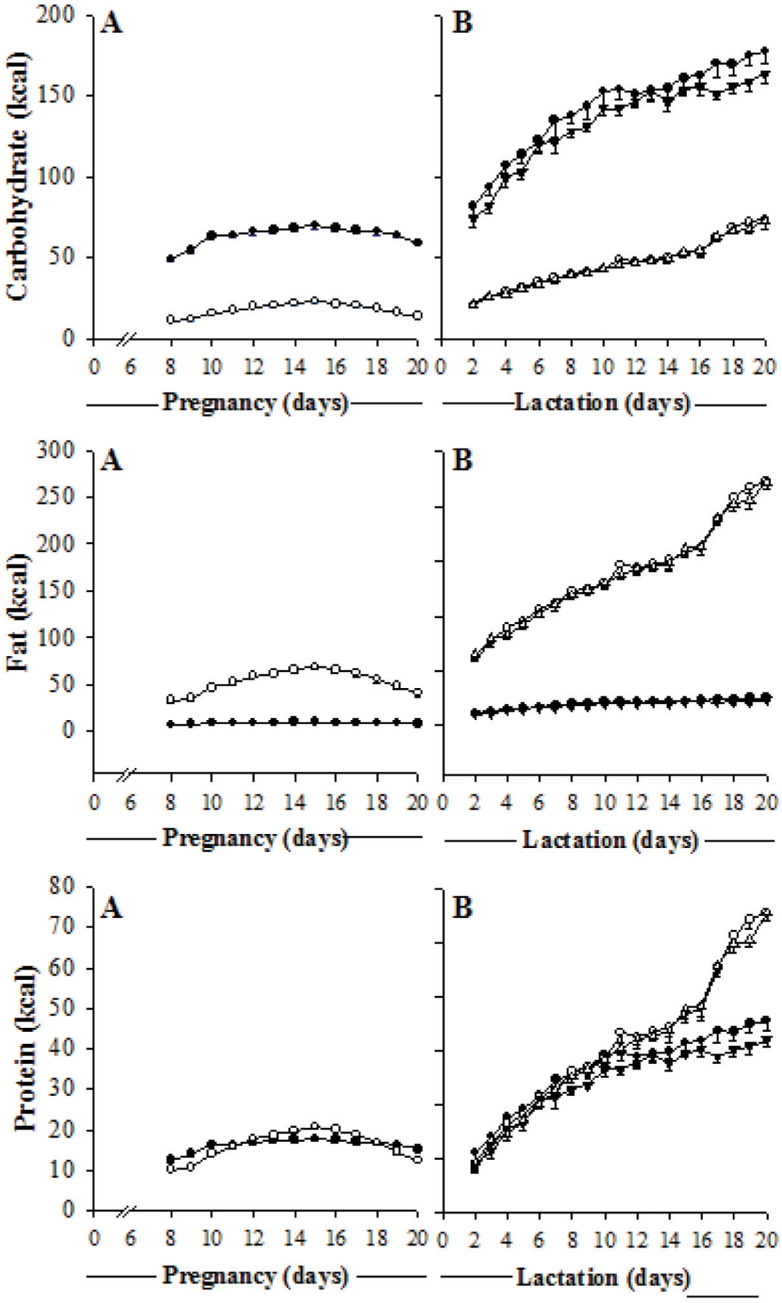

Figure 2. Calorie Intake during Pregnancy and Lactation

(A) Daily maternal calorie intake during pregnancy (e8–e20) of Con (●) and HF dams (○). (B) Daily maternal calorie intake during lactation (day 2–20) of Con dams nursing Con pups (●), high fat dams nursing high fat pups (○), Con dams nursing high fat pups (▲) and high fat dams nursing Con pups (Δ). Values are mean±SE of N=16 dams per group during pregnancy and N=8 per group during lactation.

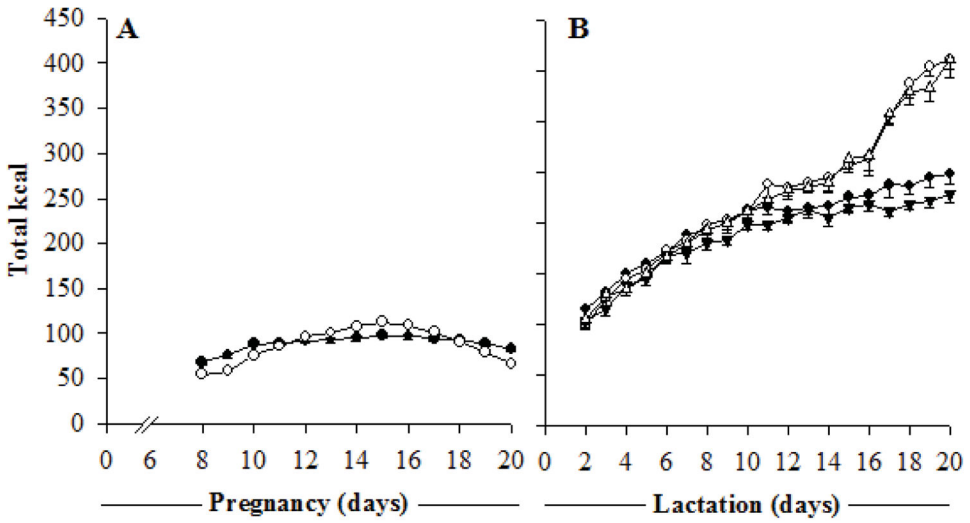

Figure 3. Total Maternal Calorie Intake during Pregnancy and Lactation

(A) Daily maternal total calorie intake during pregnancy (e8–e20) of Con (●) and HF dams (○). (B) Daily maternal total calorie intake during lactation (day 2–20) of Con dams nursing Con pups (●), high fat dams nursing high fat pups (○), Con dams nursing high fat pups (▲) and high fat dams nursing Con pups (Δ). Values are mean±SE of N=16 dams per group during pregnancy and N=8 per group during lactation.

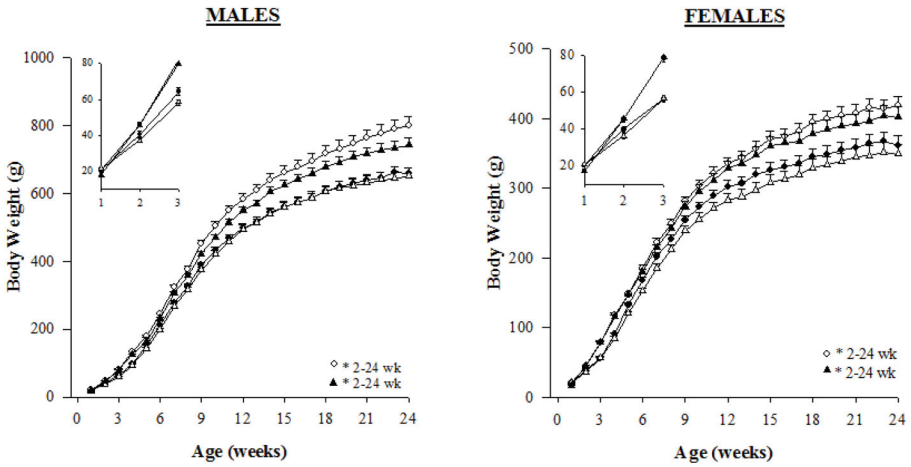

Figure 4. Offspring Body Weights

Mean body weights of male and female offspring from 1 to 24 weeks of age in Con (● pups born to Con dams and nursed by Con dams), HF/HF (○ pups born to HF dams and nursed by HF dams), HF/Con (Δ pups born to HF dams and nursed by Con dams), and Con/HF(▲ pups born to Con dams and nursed by HF dams). Insets: body weights of males and females from 1 day to 3 wk of age. Number of offspring studied per group was 32 males and 32 females (from 8 litters) until 3 wk of age, after which half the offspring were weaned onto a Con diet. Thereafter, data are shown from 16 males and 16 females (from 8 litters) until 24 weeks of age per group. Values are mean±SE; *P<0.05 vs. Con.

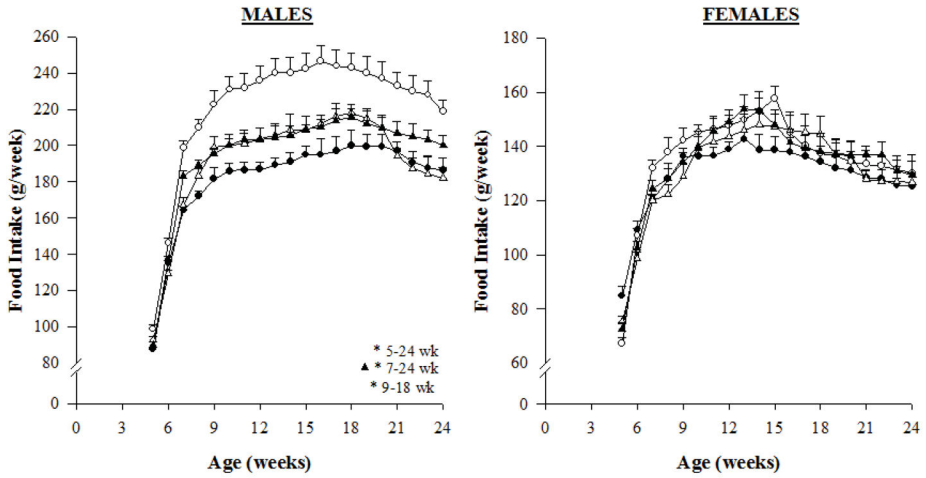

Figure 5. Offspring Food Intake

Mean food intake of male and female offspring from 5 to 30 weeks of age in Con (● pups born to Con dams and nursed by Con dams), HF/HF (○ pups born to HF dams and nursed by HF dams), HF/Con (Δ pups born to HF dams and nursed by Con dams), and Con/HF(▲ pups born to Con dams and nursed by HF dams). Number of offspring studied per group was 32 males and 32 females (from 8 litters) until 3 wk of age, after which all offspring were weaned onto a Con diet. Thereafter, data are shown from 16 males and 16 females (from 8 litters) until 30 weeks of age per group. Values are mean±SE; *P<0.05

Figure 6A

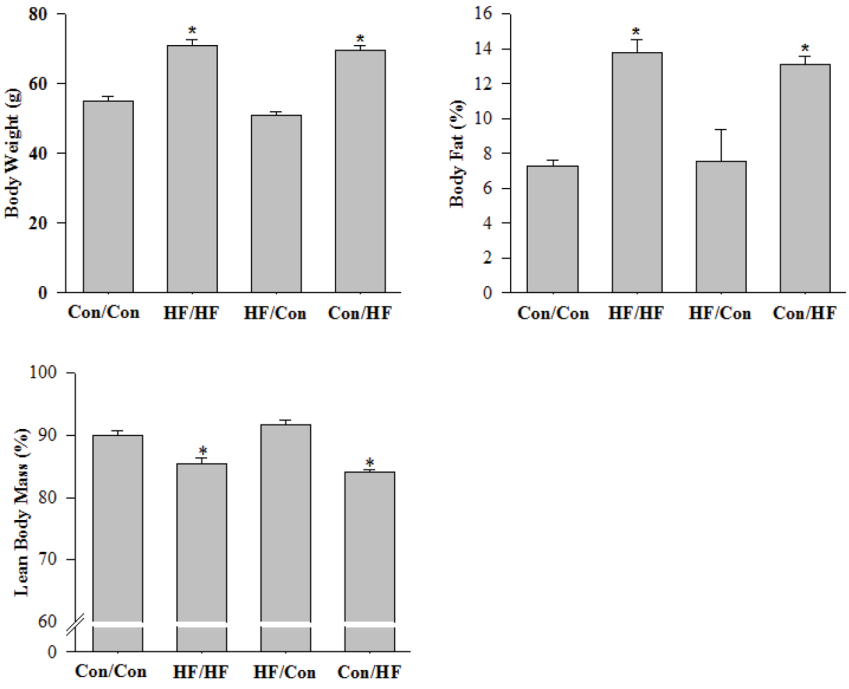

Figure 6B

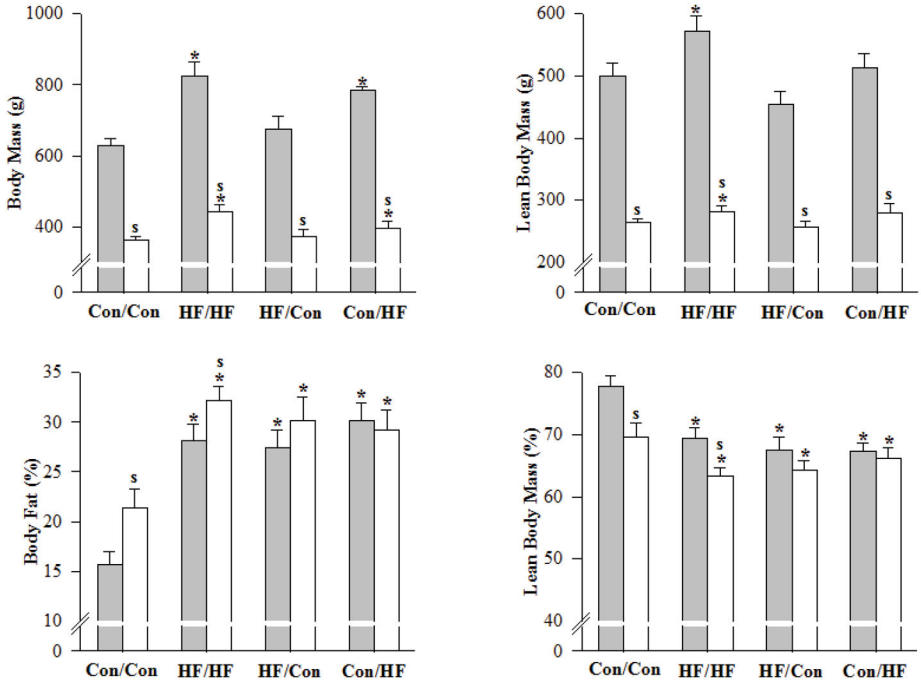

Figure 6.

Figure 6A: Body Composition of 3 Week Old Offspring

Body mass, % body fat and % lean body mass in 3 week old offspring. Because no sex differences were evident, combined data of males (8) and females (8) from 8 litters in the 4 groups are shown. Values are mean±SE; *P < 0.001 vs. Con offspring.

Figure 6B: Body Composition of 24 Week Old Offspring

Body mass, lean body mass, and percentage body fat and lean body mass in 24 week old male (■) and female (□) offspring from four groups. Number of offspring studied per group was 8 males and 8 females from 8 litters. Values are mean±SE; *P <0.001 vs. Con offspring; ^{S}P <0.001 male vs. female.

Figure 7A

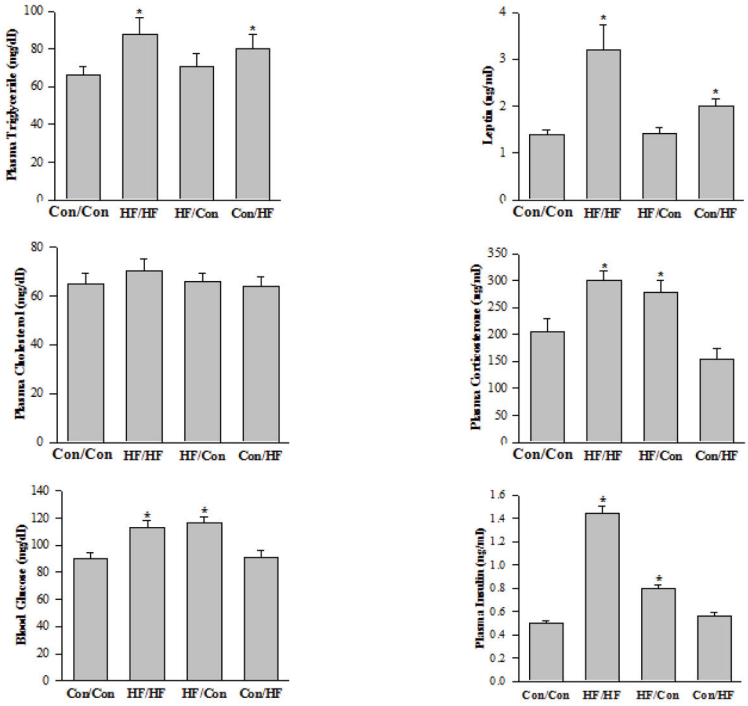

Figure 7B

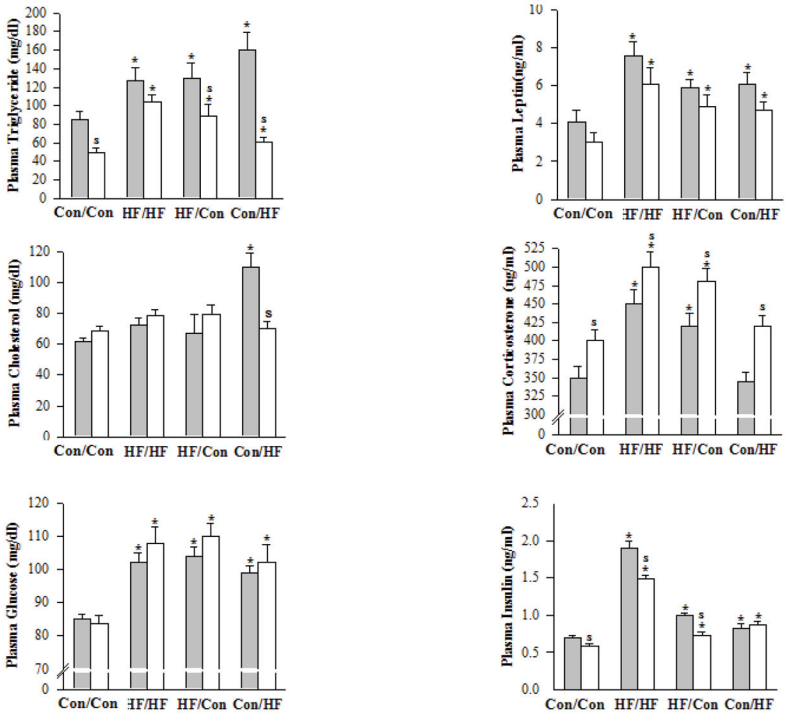

Figure 7.

Figure 7A: Plasma Profile of 3 Week Old Offspring

Plasma triglyceride, cholesterol, glucose, leptin, corticosterone and insulin in 3 week old offspring. Because no sex differences were evident, combined data of males (8) and females (8) from 8 litters in the 4 groups are shown. Values are mean±SE; *P <0.01 vs. Con offspring.

Figure 7B: Plasma Profile of 24 Week Old Offspring

Plasma triglyceride, cholesterol, glucose, leptin, corticosterone and insulin in 24 week old male (■) and female (□) offspring from four groups. Number of offspring studied per group was 8 males and 8 females from 8 litters. Values are mean±SE; *P <0.001 vs. Con offspring; §P <0.001 male vs. female

Figure 8A

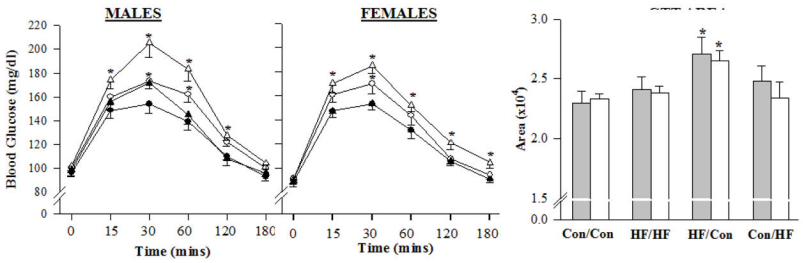

Figure 8B

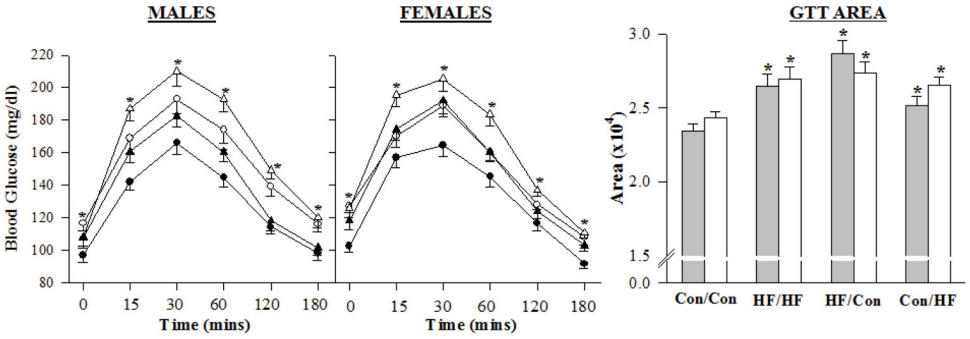

Figure 8.

Figure 8A: Glucose Tolerance Test at 6 Weeks of Age

Mean glucose values of male and female offspring at 6 weeks of age from Con (● pups born to Con dams and nursed by Con dams), HF/HF (○ pups born to HF dams and nursed by HF dams), HF/Con (Δ pups born to HF dams and nursed by Con dams), and Con/HF(▲ pups born to Con dams and nursed by HF dams). GTT area under the curve of male (■) and female (□) offspring from four groups. Number of offspring studied per group was 8 males and 8 females (from 8 litters). Values are mean±SE; *P<0.05.

Figure 8B: Glucose Tolerance Test at 24 Weeks of Age

Mean glucose values of male and female offspring at 24 weeks of age from Con (● pups born to Con dams and nursed by Con dams), HF/HF (○ pups born to HF dams and nursed by HF dams), HF/Con (Δ pups born to HF dams and nursed by Con dams), and Con/HF(▲ pups born to Con dams and nursed by HF dams). GTT area under the curve of male (■) and female (□) offspring from four groups. Number of offspring studied per group was 8 males and 8 females (from 8 litters). Values are mean±SE; *P<0.05.

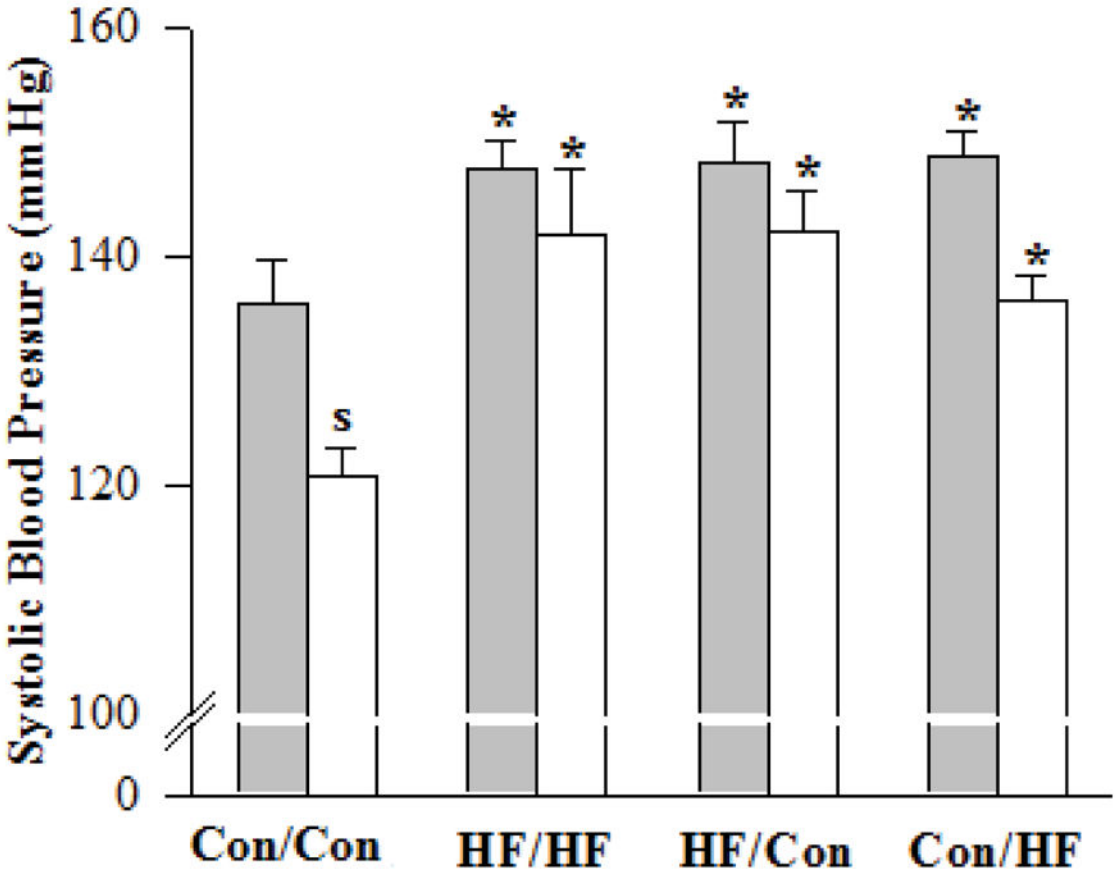

Figure 9. Systolic Blood Pressure

Systolic blood pressure in 8 week old male (■) and female (□) offspring from four groups. Number of offspring studied per group was 8 males and 8 females from 8 litters. Values are mean±SE; *P <0.001 vs. Con offspring.

Table 1

Nutrient Composition of Diets

	Purified Diet 58Y2 (10% k/cal fat)	Purified Diet 58Y1 (60% k/cal fat)
Nutrients (%)		
Carbohydrate	67.4	25.9
Protein	17.3	23.1
Fat	4.3	34.9
Lard	1.9	31.7
Soybean oil	2.4	3.2
Fat Type (%)		
Saturated	25	37
Monounsaturated	35	46
Polyunsaturated	40	17

Nutrient values are percentage per 100g food and fat type is percentage of total fat.

Table 2

Plasma Profile of Pregnant Dams

	Con	HF
Plasma triglyceride (mg/dl)	164 ± 13	196 ± 16
Plasma cholesterol (mg/dl)	110 ± 5	128 ± 3*
Blood glucose (mg/dl)	57 ± 3	63 ± 5
Plasma leptin (ng/ml)	3.3 ± 0.3	4.3 ± 0.5
Plasma corticosterone (ng/ml)	266 ± 34	776 ± 64*

Plasma lipid, glucose and hormone levels of pregnant dams at term (gestational age e20). Values are mean±SE of N=16 dams per group.

* P<0.05 vs. Con.

Table 3

Plasma Profile of Lactating Dams

	Con dams nursing Con pups	Con dams nursing HF pups	HF dams nursing HF pups	HF dams nursing Con pups
Body Fat (%)	4.6 ± 0.4	5.6 ± 0.6	12.1 ± 1.2*	7.5 ± 0.8*
Lean Body Mass (%)	92.9 ± 0.3	91.5 ± 0.6	85.4 ± 1.2*	90.0 ± 1.9
Plasma triglyceride (mg/dl)	72 ± 9	60 ± 9	43 ± 6*	45 ± 3*
Plasma cholesterol (mg/dl)	95 ± 7	91 ± 6	54 ± 5*	65 ± 2*
Blood glucose (mg/dl)	57 ± 7	53 ± 6	68 ± 7	62 ± 5
Plasma leptin (ng/ml)	1.6 ± 0.3	1.3 ± 0.2	2.1 ± 0.4*	1.6 ± 0.4
Plasma insulin (ng/ml)	0.12 ± 0.02	0.16 ± 0.02	0.23 ± 0.05*	0.18 ± 0.02*
Plasma corticosterone (ng/ml)	247 ± 47	588 ± 47*	414 ± 45*	397 ± 45*

Body composition and plasma lipid, glucose and hormone levels of lactating dams at day 21. Values are mean±SE of N=8 dams per group.

* P<0.05 vs. Con.

Table 4

Plasma Profile of Newborn Pups

	Con	HF
Body weight (g)	7.3 ± 0.1	7.4 ± 0.2
Plasma triglyceride (mg/dl)	126 ± 9	92 ± 12*
Plasma cholesterol (mg/dl)	95.7 ± 2.7	82.5 ± 3.3*
Plasma leptin (ng/ml)	4.9 ± 0.9	2.1 ± 0.5*
Plasma Insulin (ng/ml)	0.52 ± 0.10	0.52 ± 0.20
Plasma corticosterone (ng/ml)	80 ± 12	52 ± 8*

1 day old Con and HF pups at from. Because no sex differences were evident, combined data of males (16) and females (16) from 16 litters in the 4 groups are shown.

*P<0.05 vs. Con.