Maternal obesity is necessary for programming effect of high-fat diet on offspring

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White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. Am J Physiol Regul Integr Comp Physiol 296: R1464–R1472, 2009. First published February 25, 2009; doi:10.1152/ajpregu.91015.2008.—We tested the hypothesis that maternal consumption of dietary fat, independent from obesity, increases serum leptin in neonatal pups and predisposes them to adult obesity. Female rats either were fed a high-fat (HF) diet or a low-fat (LF) diet or were fed the HF diet but pair fed (PF) to the caloric intake of the LF group for 4 wk before breeding and throughout gestation and lactation. Dams consuming the HF diet had increased adiposity and were hyperphagic. At weaning, pups born to obese dams had significantly higher body fat and serum leptin levels and reduced insulin tolerance compared with offspring of LF-fed dams. Pups were weaned onto a chow diet until 8 wk of age, when they were then fed either HF or LF diet. At 18 wk of age, offspring from obese HF dams weighed more than offspring from nonobese LF or PF dams, and offspring eating HF diet weighed significantly more than those eating LF diet. Consequently, HF-fed offspring of obese HF dams weighed the most and LF-fed offspring from obese HF dams were similar in weight to HF-fed offspring from nonobese LF dams. These data suggest that maternal obesity exerts an independent effect on offspring body weight that is of similar magnitude as the effect of the offspring’s adult diet. Furthermore, there was no difference in body weight between the nonobese LF and PF offspring on either diet. Together, these data suggest that maternal adiposity, and not dietary fat per se, induces hyperleptinemia and insulin resistance in offspring, as well as an increased body weight that persists into adulthood.

MATERIALS AND METHODS

Animal maintenance and breeding. Twenty 8-wk-old virgin female Long-Evans rats (Harlan Laboratories, Indianapolis, IN) were singly housed in shoebox cages in a room with a 12:12-h light-dark cycle. Water was provided ad libitum through an automated watering system. Rats were fed either a high-fat (HF) diet or a low-fat (LF) diet or were fed the HF diet but were pair fed (PF) to the caloric intake of the LF group. The HF diet (Research Diets D12492) consisted of 60% fat energy, 20% carbohydrate energy, and 20% protein energy and had an energy density of 5.24 kcal/g. The LF diet (Research Diets D12450B) consisted of 10% fat energy, 70% carbohydrate energy, and 20% protein energy, with an energy density of 3.85 kcal/g. After 4 wk of eating these diets, rats were bred with 10-wk-old virgin male Chow-fed Long-Evans rats. Dams continued on their respective diets throughout both gestation and lactation. Food intake was measured daily in the dams.

Within 24 h of birth, litter size was adjusted to 10 pups per litter and sexes were distributed as equally as possible (HF 5.2 ± 0.8, LF 5.5 ± 0.3, and PF 5.2 ± 0.7 males/litter). At 3 wk of age, one male pup/litter was euthanized by decapitation and trunk blood and brain and adipose tissue were collected. The remaining male pups were weaned onto chow and multihoused in shoebox cages until 8 wk of age. Because of the possibility of sexual dimorphism, only male pups were utilized in the studies reported in this article.

At 8 wk of age, pups from each litter were divided into two groups and fed either a HF or a LF diet until 20 wk of age. At that time, rats were euthanized and trunk blood and brain and adipose tissue were collected. The Institutional Animal Care and Use Committee approved all of the experimental protocols, which were consistent with National Institutes of Health guidelines on the use of experimental animals.

Body weight and body composition analysis. Body weight was measured weekly in the dams before conception and during gestation. During lactation, both the dams and the pups were weighed on days 2, 7, 14, and 20 postpartum. Pups were then weighed weekly throughout the remainder of the study. Body composition was measured in the
then stored at -80°C. Milk samples were expressed and collected into a Pasteur pipette. Milk samples were peritoneally with 4 IU of oxytocin (Parkedale Pharmaceuticals, Rochester, MI) to stimulate milk flow. Five minutes after oxytocin injection, milk was expressed and collected into a Pasteur pipette. Milk samples were then stored at -20°C until analysis. Blood was also collected at this time, via the lateral tail vein.

**Milk and blood collection from dams.** Milk was collected from the dams at 7 days postpartum. Pups were separated from the dams for 30 min. Dams were then anesthetized with isoflurane and injected intraperitoneally with 4 IU of oxytocin (Parkedale Pharmaceuticals, Rochester, MI) to stimulate milk flow. Five minutes after oxytocin injection, milk was expressed and collected into a Pasteur pipette. Milk samples were then stored at -20°C until analysis. Blood was also collected at this time, via the lateral tail vein.

**Insulin tolerance test.** An insulin tolerance test (ITT) was performed on the offspring at weaning, at 8 wk of age, and at 18 wk of age. Two rats from each litter received a dose of 0.5 U/kg body mass of a 50% glucose solution intraperitoneally after an 4-h fast. When tested at 18 wk of age, there was both a rat eating high fat and a rat eating low fat from each litter. Blood samples were collected from the tail of lightly restrained animals and analyzed with a commercially available glucometer (AccuCheck Advantage, Roche, Mannheim, Germany). Samples were drawn immediately before injection of insulin and at 30-min intervals for 2 h after injection.

**Intraperitoneal glucose tolerance test.** An intraperitoneal glucose tolerance test (IPGTT) was performed on the offspring at 20 wk of age. Two rats from each litter, one on each diet, received a dose of 2.5 mg/kg body mass of a 50% glucose solution intraperitoneally after an overnight fast. Blood samples were collected from the tail of lightly restrained animals and analyzed with a commercially available glucometer (AccuCheck Advantage, Roche). Samples were drawn immediately before injection of insulin and at 30-min intervals for 2 h after injection.

**Assays.** Trunk blood was allowed to clot at 4°C overnight and then centrifuged at 3,000 g for 30 min, and serum was collected and stored at -80°C. Serum leptin was analyzed with a radioimmunoassay kit (Linco Research, St. Louis, MO). Glucose was measured on an AccuCheck Advantage glucometer (Roche). Adipocyte cell size and number. A subset of five rats/group was used to measure adipocyte cell size and number as described by Smith and colleagues (3). Fifty milligrams of the retroperitoneal fat pad was washed in saline and then fixed in osmium tetroxide solution (31 mg/ml of 0.2 M collidine HCl buffer). At the time of analysis, samples were diluted with 154 mM NaCl and dissociated over 1 wk by the addition of 10 mL of 8 M urea in 154 mM NaCl. The sample was then filtered through a 250-μm nylon filter into a weighed beaker. The volume was increased to 300 ml with 154 mM NaCl containing 0.1% Triton X-100. The cells were counted on a Multisizer-3 (Beckman Coulter, Fullerton, CA) using a 400-μm aperture (dynamic linear range 12–320 μm) and presented as the geometric mean.

**Data analysis.** Data were analyzed by two-way ANOVA via the general linear model (GLM) procedure in the SAS software package (SAS V8, SAS Institute, Cary, NC). When experiment-wide tests for the main effects of maternal diet, offspring diet, or their interaction were significant, post hoc comparisons were made with the LSMEANS statement with the PDIF option and thus represent least significant difference tests for preplanned comparisons. All data are expressed as means ± SE, with a probability value of <0.05 considered statistically significant.

**Fig. 2.** Effect of HF diet on dams during lactation. Female Long-Evans rats were fed a HF diet or a LF diet or were pair fed (PF) to the caloric intake of the LF group for 4 wk and then bred, continuing on their respective diets throughout lactation. A: serum and milk were collected at postpartum day 7, and leptin levels were measured. Values are expressed as mean ± SE leptin values, with 3–6 animals per group. B: serum glucose was measured at weaning. Values are expressed as mean ± SE glucose values, with 3 or 4 animals per group. *Difference from LF group, P < 0.05.
RESULTS

Dam body weight, food intake, and body composition analysis. Female rats were fed a HF or LF diet for 4 wk before breeding and continued on this diet throughout gestation and lactation. By 2 wk on the HF diet the HF group weighed significantly more than the LF and PF groups, and this difference continued through the end of lactation (Fig. 1). HF rats also had a higher average daily food intake (71.24 ± 3.59 kcal/day, \( n = 7 \)) during the 4 wk of diet before breeding compared with LF (56.61 ± 1.86 kcal/day, \( n = 7; P < 0.0005 \)) and PF (56.00 ± 0.54 kcal/day, \( n = 6; P < 0.0005 \)) rats.

Maternal obesity had significant effects on both serum and milk leptin during lactation. There were significant differences between the obese HF group and both the nonobese LF and PF groups in serum leptin at day 7 and a significant difference between the LF and HF groups in milk leptin (Fig. 2). By day 21, there was no difference in serum leptin between LF (2.59 ± 0.33 ng/ml, \( n = 4 \)) and HF (2.79 ± 0.46 ng/ml, \( n = 4; P = 0.5 \)) groups, but there was still a significant difference between the HF and PF (0.99 ± 0.28 ng/ml, \( n = 3; P = 0.01 \)) groups. At day 21 there was also a difference between the LF and PF groups (\( P = 0.05 \)). Serum glucose levels are also shown in Fig. 2; there were no significant differences between the groups at day 21.

Offspring adiposity and insulin responsiveness from birth to weaning. Differences in adiposity in the pups from birth through weaning are shown in Fig. 3. There were no differ-

![Graphs showing changes in body weight, serum leptin, and retroperitoneal fat pad weight from birth to weaning](http://ajpregu.physiology.org/)

**Fig. 3.** Measures of adiposity from male offspring of dams fed HF or LF at weaning. Female Long-Evans rats were fed a HF diet or a LF diet or were pair fed (PF) to the caloric intake of the LF group for 4 wk and then bred, continuing on their respective diets throughout lactation. A: offspring body weight from birth until weaning of 26–34 animals per group. B: serum leptin levels measured at weaning of male offspring; 5 or 6 animals per group. C: weight of retroperitoneal fat pad expressed as mean ± SE % of body weight; 5 or 6 animals per group. D: mean ± SE % of body fat per gram of body weight measured via NMR at weaning; 22–25 animals per group. *Difference from LF group, \( P < 0.05 \).
ences in birth weight or litter size (data not shown). By 7 days of age pups born to maternally obese HF dams weighed significantly more than those born to nonobese LF or PF dams, and this difference continued to diverge through weaning. Serum leptin levels were significantly higher in pups from obese HF-fed dams than in pups from nonobese LF- or PF-fed dams. As with body weight and serum leptin, retroperitoneal fat pads and whole body adiposity (NMR) were significantly higher in pups from an obese HF-fed dam compared with both nonobese LF- and PF-fed dams (Fig. 3). Additionally, the offspring of the PF group had a significantly higher percentage of body fat than the offspring of the LF group, as measured by NMR.

Insulin sensitivity was measured in the pups on the day of weaning via ITT (Fig. 4). Pups from obese HF-fed dams exhibited a reduced insulin tolerance compared with pups from nonobese LF or PF dams, with pups from PF dams exhibiting a response that was intermediate between the LF and HF groups. However, baseline serum glucose levels were not different between offspring of nonobese LF dams (117.6 ± 3.4 mg/dl, n = 12) and either the HF (113.5 ± 3.2 mg/dl, n = 10) or PF offspring (125.3 ± 3.7 mg/dl, n = 9).

Offspring adiposity, food intake, and insulin responsiveness while eating chow. After weaning, the offspring were fed a chow diet for 5 wk. Offspring of obese HF-fed dams weighed significantly more at weaning, and this difference persisted for the 5-wk period (Fig. 5). However, there was no difference in the rate of body weight change between the groups during this time (LF 205.1 ± 4.2 g, n = 20; HF 216.3 ± 5.8 g, n = 20; PF 209.7 ± 3.5 g, n = 20; P = 0.235). There was also no difference in average daily food intake between the groups (Fig. 5). NMR performed after 5 wk of chow indicated that offspring from obese HF dams had a significantly higher percentage of body fat (Fig. 5). However, an ITT performed at this time suggests no significant difference in the baseline glucose values or area under the curve (Fig. 5).

**Phenotype of adult offspring.** After 5 wk of chow intake (8 wk of age), offspring were assigned to either a HF or LF diet. During this period, body weights progressively diverged such that pups born to obese HF dams and consuming a HF diet weighed significantly more than any other group, whereas pups born to nonobese LF or PF dams and consuming LF weighed the least (Fig. 6). Intermediate between these groups were those pups that either were born to obese HF mothers or consumed HF in adulthood. Statistically, body weight was significantly affected by maternal obesity (P < 0.01) and offspring diet (P = 0.005), but the interaction between these effects was not significant, indicating that the effects were independent and additive. Similarly, there was a significant main effect of maternal obesity (P < 0.05) and offspring diet (P < 0.005) on cumulative food intake, but no interaction (P = 0.97). Feed efficiency (gain/feed) was also analyzed, and the results are in Table 1. All of the offspring eating a HF diet had a significantly higher feeding efficiency than their maternal diet counterpart eating a LF diet.

In addition to body weight, there were significant differences in body adiposity in the offspring after eating a HF or LF diet (Fig. 7), with a significant main effect of maternal obesity (P < 0.01) and of offspring diet (P < 0.005) but no interaction between the two. In the offspring of the obese HF-fed dams, there a significant difference in percent body fat between the offspring eating a HF and LF diet. Offspring of obese HF-fed dams eating a HF diet had significantly higher percent body fat than offspring of either nonobese LF or PF dams eating a HF diet. Significant main effects of maternal obesity (P < 0.005) and offspring diet (P < 0.0001) were also detected for body adiposity, as measured by retroperitoneal fat pad weights (Table 1). Fat pad weights were significantly higher in offspring eating a HF diet compared with offspring eating a LF diet within the same maternal diet group, while offspring of obese HF dams eating a HF diet also had higher fat pad weights than offspring of nonobese LF dams eating a HF diet. There was no interaction between maternal obesity and offspring diet on retroperitoneal fat pad weights, again indicating that the two main effects interacted additively. Adipocyte cell size and number were also measured in the retroperitoneal fat pad (Table 1). There was no effect of either maternal obesity or offspring diet on adipocyte number, nor was there an effect of maternal obesity on adipocyte cell size. However, there was a significant effect of offspring diet on mean cell size, with offspring of nonobese LF fed dams eating a HF diet having a larger mean cell size than those eating a LF diet. Serum leptin levels were not significantly different between groups (Table 1).

After the offspring had been on their respective LF or HF diet for 10 wk, an ITT was performed. There were no differences in baseline glucose, nor was there a significant effect of maternal obesity or offspring diet on insulin sensitivity (data not shown). However, there was a trend toward an effect of maternal obesity (P = 0.11), an IPGTT was performed. Again, there was no difference in baseline glucose...
and no effect of either maternal obesity nor offspring diet but a trend toward an effect of maternal obesity was detected. (Fig. 8).

After the offspring were killed at 20 wk of age, hypothalamic RNA was isolated and expression of NPY, AGRP, and POMC was analyzed (Table 1). Levels of hypothalamic NPY were higher in LF-fed offspring of obese HF-fed dams compared with both offspring of LF and PF dams. They were also higher compared with HF-fed offspring of obese HF-fed dams. There were no differences in hypothalamic AGRP expression. There was a significant main effect of offspring diet for hypothalamic POMC, but there were no significant individual differences of interest.

RNA was also isolated from BAT and levels of UCP1 and β3AR were analyzed (Table 1). Offspring of obese HF-fed dams that were eating a LF diet had higher levels of β3AR expression than offspring of nonobese LF-fed dams that were eating a LF diet. Likewise, in offspring eating a HF diet, those born to obese HF-fed dams had higher β3AR expression than those born to nonobese LF-fed dams. UCP1 expression was not affected by maternal diet but was affected by offspring diet. All of the pups eating a HF diet had significantly higher levels of UCP1 than their maternal diet counterparts eating a LF diet.

### DISCUSSION

The present experiments confirm previous studies indicating that consumption of a high-fat diet before and during pregnancy leads to obesity in the adult offspring of that pregnancy. In addition, the present work extends these observations by demonstrating that it is maternal obesity and not the consumption of dietary fat per se that is the primary mechanism driving offspring obesity. When mothers were fed a HF diet but restricted to the caloric intake of the LF group (PF), they failed to become obese and this prevention of maternal obesity resulted in normal body weight homeostasis in their adult offspring. Contrastingly, ad libitum maternal consumption of the HF diet resulted in obese dams whose offspring weighed more by 7 days of age. This trend continued throughout the remainder of the study, such that these offspring were heavier in adulthood than offspring of nonobese dams whether they were fed a LF or a HF diet. Maternally obese dams had higher levels of leptin both in serum and in milk during lactation than nonobese dams. Offspring of obese dams were also less sensitive to insulin at weaning than offspring of nonobese dams, although this effect was lost by 8 wk of age. Perhaps most remarkable, our data indicate that exposure to maternal obesity produces an increase in body weight that is similar in magnitude to the effect of the offspring actually eating a HF diet in
adulthood. These effects of maternal obesity and offspring HF diet acted additively, such that getting a single exposure (either in utero or in adulthood) produced an increase in body weight, while being exposed to both resulted in a further increase in body weight and adiposity.

Increased body weight in the offspring of obese dams at weaning appears to be due to an increase in body fat, because results from both the NMR and retroperitoneal fat pad weights indicate increased adiposity but no difference in lean mass. The increase in body weight and body fat is still seen at 8 wk of age, despite the offspring being maintained on chow for 5 wk after weaning. Thus maternal obesity produces an increase in body weight and adiposity at weaning that persists well into adulthood. When the offspring are then placed on LF or HF diets, the groups diverge and a clear pattern emerges. In this pattern, the lightest offspring are those that are exposed to neither maternal obesity nor the HF diet in adulthood (offspring from nonobese LF and PF dams and eating a LF diet). Contrastingly, the heaviest group is those offspring that were both born to obese mothers and actively eating the HF diet. These offspring in essence receive a double dose, once from their mothers and once in adulthood. Finally, the remaining groups stratify to a weight that is intermediate between these extremes. These offspring were either born to an obese mother and ate LF in adulthood or were born to a nonobese mother but were placed on the HF diet in adulthood. Thus these intermediate groups reflect offspring that, in effect, only received a single dose of the HF diet. Most interestingly, these data indicate that maternal obesity produces an increase in body weight that is similar in magnitude to the increase that is induced by HF diet consumption in adulthood. Thus the offspring that were born to an obese mother were as heavy as those eating a HF diet, even though the former group was never exposed to a HF diet in adulthood.

While other studies have demonstrated that maternal obesity or maternal fat intake programs obesity in the offspring (5, 26, 40, 43), not all work leads to the same conclusions. In one case a high-fat diet was fed to dams without observing any effect of body weight in the offspring (11), while other studies either have not seen a difference at weaning or have observed a lower body weight at weaning in offspring of high-fat-fed mothers (6, 39, 40). However, our data replicated other studies showing an increase in body weight at weaning of high-fat-fed offspring (16, 21). The varied results from different studies can be explained by the use of different fat sources (lard, vegetable oil, cafeteria diet, margarine, etc) and the different times and
The increase in body weight in offspring of obese dams could reflect increased food intake or decreased energy expenditure. Food intake was significantly increased in the offspring of obese dams and, as such, likely contributed to their increased weight gain. Unfortunately, the data from BAT do not provide such a clear picture. BAT thermogenesis, which is reduced in obesity, is a major component of energy expenditure. The higher feed efficiency observed in offspring of obese dams compared with those eating a LF diet, maternal obesity does not follow the same pattern. Offspring of the obese dams eating a HF diet have significantly higher levels of BAT β3AR than the offspring of dams eating a LF diet. Even levels of BAT β3AR in offspring of the PF dams who were eating a HF diet but were nonobese are higher, though not significantly, than those of offspring of the LF rats. These results would suggest a higher level of sympathetic activity in the offspring of dams eating a HF diet. However, results of UCP1 message in BAT do not support a higher level of sympathetic activity in offspring of HF-fed dams, because maternal obesity had no effect on UCP1 levels. Additionally, offspring eating a HF diet had significantly higher levels of BAT UCP1, which was also unexpected and does not match the feeding efficiency data. Ultimately, these differences will be best studied with a metabolic chamber, which we hope to do in the future.

Increases in body adiposity can be due to an increase in adipocyte size and adipocyte cell number. Increased cell size is generally believed to be the most important factor in determining obesity (8, 23). However, there is evidence that increased length of time that the dams are exposed to the diets, thus illustrating the possibility of several developmental windows.
cell number is associated with an increased predisposition to obesity (22). Furthermore, recent work by Spalding et al. (42) shows not only that fat cell number is an important determinant in adult obesity but also that this number is fixed during childhood. We therefore chose to assess both cell size and number in retroperitoneal fat pads, to specifically test the hypothesis that maternal obesity might have a programming effect on adipose tissue, inducing hyperplasia that could predispose offspring to obesity. Interestingly, we observed no effect of maternal obesity on either adipocyte size or number. Work by Caluwaerts et al. (12) did show an effect of maternal obesity on cell size at weaning but not at 8 wk of age. Thus these data are not consistent with the hypothesis that maternal obesity programs an increase in adipocyte number. Consistent with previous reports (2, 36, 40), we did detect an effect of offsprings HF diet consumption on adipocyte size. There was no effect on cell number, but results differ in the literature about the effect of a HF diet on hyperplasia (2, 19, 28, 33, 40). Because rat strain and fat cell depot examined also have an effect on cell size (35), these strain and depot variations could account for the variable results.

The present work provides evidence that supports a model in which the obese maternal environment produces permanent programming effects on the offspring. Yet the specific components of the obese maternal environment that produce these changes are not fully clear. In rodents, the central nervous system continues to develop during the first few weeks of postnatal life. Included in this process are leptin-sensitive arcuate nucleus neurons, whose projections to downstream brain areas mature within the first 2 wk of birth (9). Leptin replacement during the first 2 wk of life is sufficient to permanently rescue underdeveloped hypothalamic architecture in ob/ob mice, indicating that leptin action during this critical period permanently organizes the structure of neuronal pathways regulating energy homeostasis (9). Offspring from HF dams in the present work exhibited significantly increased circulating leptin levels, and these altered leptin levels are thus positioned to influence the structure of these neuronal pathways. The most likely mechanism driving these increases in circulating leptin is the increased body adiposity of the pups themselves. However, obese HF dams also exhibited increased levels of leptin within milk. Considering that leptin given orally to neonates is absorbed into the bloodstream (13), and that leptin given to dams during lactation or gestation influences weight gain in the offspring (30, 44), it remains possible that variations in milk leptin levels could contribute to alterations in circulating leptin in the pups. Regardless of the mechanism, these data clearly indicate that pups born to HF-fed, obese mothers exhibit increased levels of circulating leptin, consistent with the possibility that this hyperleptinemia induces permanent changes in the development of leptin-sensitive circuits within the brain.

**Perspectives and Significance**

The work described here supports the concept of nutritional programming and indicates that offspring of maternally obese dams weigh more and exhibit increased body adiposity compared with offspring of nonobese dams. In addition, the present work extends this observation by effectively distinguishing between the direct effects of a high-fat diet and effects that are secondary to maternal obesity. Because the offspring of HF-fed nonobese (PF) dams were similar to the offspring of LF dams, these data clearly indicate that consumption of a high-fat diet itself (i.e., in the absence of obesity) is insufficient to predispose offspring to obesity. It is instead maternal obesity that is required for this programming effect, indicating that some feature of the obese maternal environment induces a permanent structural or functional change in the offspring, with this change predisposing the offspring to obesity.

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**GRANTS**

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