Maternal obesity at conception programs obesity in the offspring

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1Arkansas Children’s Nutrition Center, Little Rock; and Departments of 2Pharmacology and Toxicology, 3Physiology and Biophysics, and 4Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

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Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. Am J Physiol Regul Integr Comp Physiol 294: R528–R538, 2008. First published November 21, 2007; doi:10.1152/ajpregu.00316.2007.—Risk of obesity in adult life is subject to programming during gestation. To examine whether in utero exposure to maternal obesity increases the risk of obesity in offspring, we developed an overfeeding-based model of maternal obesity in rats utilizing intrauterine feeding of diets via total enteral nutrition. Feeding liquid diets to adult female rats at 220 kcal/kg3/4 per day (15% excess calories/day) compared with 187 kcal/kg3/4 per day for 3 wk caused substantial increase in body weight gain, adiposity, serum insulin, leptin, and insulin resistance. Lean or obese female rats were mated with ad libitum AIN-93G-fed male rats. Exposure to obesity was ensured to be limited only to the maternal in utero environment by cross-fostering pups to lean dams having ad libitum access to AIN-93G diets throughout lactation. Numbers of pups, birth weight, and size were not affected by maternal obesity. Male offspring from each group were weaned at postnatal day (PND)21 to either AIN-93G diets or high-fat diets (45% fat calories). Body weights of offspring from obese dams did not differ from offspring of lean dams when fed AIN-93G diets through PND130. However, offspring from obese dams gained remarkably greater (P < 0.005) body weight and higher %body fat when fed a high-fat diet. Body composition was assessed by NMR, X-ray computerized tomography, and weights of adipose tissues. Adipose histomorphometry, insulin sensitivity, and food intake were also assessed in the offspring. Our data suggest that maternal obesity at conception leads to fetal programming of offspring, which could result in obesity in later life.

PREVALENCE OF OVERWEIGHT and obesity in the United States continues to rise steadily with one in two adults being overweight [body mass index, (BMI) ≥ 25] and as many as one in five adults (~60 million individuals) being obese (BMI ≥ 30) (14, 35). Prevalence of overweight among children has nearly doubled in the last two decades (33). Perhaps more alarming is the steady increase in the risk of overweight even among infants (0–11 mo) (32, 42). This may have critical implications, as overweight in infancy and childhood significantly increases the risk of obesity in adulthood (32, 33, 42). Consistent with greater numbers of overweight individuals in the population is a remarkable rise in the incidence of obesity among pregnant women (1, 9, 10, 36, 56). Pregadiv weight is one of the most common high-risk obstetric conditions (8, 25), imparting increased risk of gestational diabetes mellitus (GDM), pregnancy-related hypertension, preeclampsia, neonatal death, and other labor complications (8, 9, 25, 36).

While much attention is paid to obesity-related complications during pregnancy, not much is known about the subtle effects of the obese intrauterine environment on the immediate and long-term health of the offspring. Data from 287,213 pregnant women clearly reveal that overweight/obese women are more likely to give birth to heavier babies (>90th percentile), consistent with the data that maternal obesity per se, even after controlling for gestational diabetes, is associated with twice higher incidence of heavier babies compared with nonobese women (8, 27, 30, 41, 44). Data from the Center for Disease Control Pediatric Nutrition Surveillance survey suggest that the odds ratios for obese children growing into obese adults are 2.1 to 8.8 times that of nonoverweight children (32). Hence, greater body weight at birth and weight gain early in life clearly increases the risk of becoming overweight or obese as an adult. This suggests that prepregnancy overweight and gestational obesity may lead to a self-reinforcing vicious cycle of excessive weight gain and adiposity that is passed on from mother to successive offspring. While the underlying mechanisms of such maternal obesity-induced programming remain unclear, the hypothesis has important implications in explaining the rapid rise in obesity.

Studies of inheritance unambiguously show BMI of children correlate more closely with maternal BMI than paternal BMI, suggesting that in addition to the genetic influences, the maternal in utero environment may contribute to the development of obesity in the offspring (12, 54). Hence, developing animal models to precisely delineate in utero influences in metabolic programming of obesity is critical to our understanding of underlying mechanisms of how increased susceptibility is passed onto the offspring. In the present report, we examined effects of obesity specifically at conception on the programming of obesity in the offspring. We have controlled for direct maternal and paternal genetic influences by developing a model of overnutrition-driven maternal obesity so as to examine only the metabolic effects of obesity on offspring health. Furthermore, by use of cross-fostering, we have excluded direct influences of maternal obesity on nursing offspring. Our data strongly suggest that exposure to maternal obesity in utero leads to metabolic programming, which results in increased susceptibility to obesity in the offspring.

MATERIALS AND METHODS

Animals and chemicals. Female Sprague-Dawley rats (150–175 g) were obtained from Charles River Laboratories (Wilmington, MA).

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Animals were housed in an American Association for Accreditation of Laboratory Animal Care-approved animal facility. Protocols for animal maintenance and experimental treatments were conducted in accordance with the ethical guidelines for animal research established and approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Unless otherwise specified, all chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

**Experimental protocol.** Virgin female Sprague-Dawley rats were intragastrically cannulated and allowed to recover for 10 days as previously described (3, 5, 6, 26, 45, 46). Rats were fed a liquid diet [20% protein (casein), 35% carbohydrate (dextrose and maltodextrin), and 45% fat (corn oil)] at National Research Council recommended caloric intake of 187 kcal/kg\(^{3/4}\) per day (referred to as lean dams) or were overfed an obesegenic liquid diet (20% protein, 75% carbohydrate, 5% fat) at 220 kcal/kg\(^{3/4}\) per day (15% excess, referred to as obese dams). Diets met caloric and nutritional guidelines recommended by the National Research Council, have been described previously (3, 5, 6), and have been utilized by our group in a number of studies (4, 19, 26, 43, 46). In preliminary experiments, we observed that while feeding at 187 kcal/kg\(^{3/4}\) per day mirrored body weights of ad libitum-fed controls (data not shown), feeding at 220 kcal/kg\(^{3/4}\) per day led to substantial obesity. Infusion of liquid diets was carried out using computer-controlled syringe pumps for 23 h/day for 3 wk. Body weights were monitored three times a week throughout the infusion period. Animals had ad libitum access to drinking water. At the end of 3 wk of infusion, body composition was noninvasively estimated using NMR (EchoMRI; Echo Medical Systems, Houston, TX) and X-ray computer tomography (X-ray CT, LaTheta LCT-100, Echo Medical Systems, Houston, TX) as detailed below. In a separate set of animals (n = 5 per group), fasting and fed levels of serum glucose, insulin, leptin, nonesterified fatty acids (NEFA), and triglycerides were assessed. In addition, insulin sensitivity was examined following an oral glucose tolerance test (OGTT) as described in *Serum hormones and OGTT.*

To examine the long-term gestational effects of maternal obesity on the offspring, lean (n = 7) and obese rats (n = 15) were allowed to mate with male rats for a period of 1 wk. At the time of mating (i.e., after 3 wk of infusion), body weights clearly diverged (obese > lean). Body composition was measured using NMR and showed the same trend as body weights. Each female rat was housed with one male and allowed ad libitum access to AIN-93G diet for this period. Following mating, all female rats (lean and obese) received their respective diets at 220 kcal/kg\(^{3/4}\) per day at the National Research Council-recommended caloric intake for pregnancy in rats. Body weights were monitored three times a week. All rats were allowed to give birth naturally. Numbers and sex of pups, birth weight, crown-to-rump and anogenital distance were measured for each pup on postnatal day (PND) 1. On PND2, four males and four female pups from each litter were cross-fostered to dams that had been previously time-impregnated to give birth on the same day as the dams receiving infusion diets. Cross-fostered dams were not cannulated and had ad libitum access to AIN-93G pellets during lactation. Using this experimental paradigm, we ensured that offspring exposure to obese-genic influences was limited only to the maternal in utero environment. Female offspring of lean and obese dams were used for separate experiments and only data from male offspring are reported here. Male offspring from each dam group (n = 7–11 per group) were weaned at PND21 to either regular AIN-93G diets (17% fat calories, caloric density 3.8 kcal/g) or to high-fat diets (HFD; 42% fat calories, caloric density 4.5 kcal/g) to mimic a postnatal obesogenic environment. Body weights of the offspring were monitored weekly until PND130. Body composition (NMR) was measured once a month throughout the study. We also assessed body composition using X-ray CT scanning at PND120. Food intake was assessed at PND60 and PND120 over a two consecutive days and average caloric intake over 24 h was calculated. OGTT was assessed in a subset of offspring (n = 5 per group) at PND120 following an overnight fast as described below. Offspring were killed at PND130 and retroperitoneal, perirenal, gonadal adipose tissue samples were collected for histology and were flash frozen for subsequent analyses. Weights of organs and adipose tissues were collected. Serum levels of glucose, triglyceride, NEFA, and other hormones were assessed as described below. Adiponectin and resistin were assayed using commercially available ELISA (B-Bridge International, Sunnyvale, CA). Total lysates from retroperitoneal adipose tissues were prepared in RIPA buffer (25 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 1% deoxycholic acid, 0.1% SDS, and 2 mM EDTA) containing 1 mM PMSF and protease inhibitor cocktail (Sigma). Proteins (20 μg) were resolved on a 10% SDS-PAGE gel and transferred to nitrocellulose membranes. Immunoblotting was carried out using standard procedures. Membranes were incubated with anti-rabbit peroxisome proliferator-activated receptor-γ (PPAR-γ) mAB (Cell Signaling Technologies, Danvers, MA) in Tris-buffered saline with 0.05% Tween-20 containing 2.5% milk for 1 h at 4°C. Following incubation with HRP-conjugated secondary IgG, membranes were washed with TBST, and proteins were visualized using West Pcco enhanced chemiluminescence kit (Pierce, Rockford, IL) and detected by autoradiography. Immunquantitation was performed by densitometric scanning of the resulting autoradiograms using a molecular imager (model GS700; Bio-Rad, Richmond, CA).

**Body composition analyses.** Body composition was assessed via three independent methods, namely, whole animal body composition by NMR (Echo Medical Systems, Houston, TX), computerized tomography (X-ray CT scanning; LaTheta LCT-100; Echo Medical Systems, Houston, TX), and postmortem dissected weights of retroperitoneal, perirenal, and gonadal fat pads from rats. Echo NMR is a quantitative magnetic resonance body composition analyzer that utilizes the resonance energy of hydrogen nuclei in a magnetic field to compute the density of the tissue. NMR is performed in conscious unanesthetized rats, and, unlike dual-energy X-ray absorptiometry, the NMR measurements are radiation-free and do not require the animals to remain still. Each NMR measurement takes ~1 min/rat, and all measurements were performed in duplicate. Indices of %fat and lean mass were derived using this technique. For CT analyses, ~90 sections, 1 mm apart, were acquired encompassing the entire visceral region of the animal under isoflurane anesthesia. Approximate time for acquisition of each slice is 4.5 s. Densitometric calculations of fat and muscle were performed using CT software (Aloka, Tokyo, Japan) using attenuation number thresholds of ~120 to ~500 for fat and ~120 to 350 for muscle. Indices of %fat ratio (ratio of volume occupied by fat/volume occupied by lean tissue), %fat mass, and

![Fig. 1. Body weights of female rats fed diets via total enteral nutrition (TEN) at 187 kcal/kg\(^{3/4}\) per day (lean, n = 7) or 220 kcal/kg\(^{3/4}\) per day (obese, n = 15) for 21 days prior to mating. Infusion of diets was carried out for 23 h/day via computer-controlled syringe pumps. Data are expressed as means ± SE. Statistical differences were determined using a Student’s t-test. *P < 0.001 compared with lean rats.](http://ajpregu.physiology.org/content/images/2017/10/201701111231426220334.png)
%lean mass were calculated. Subcutaneous and visceral fat tissues were distinguished via manual tracing of the abdominal wall in each of the sections. Serial images collected via CT analyses were collated, and solid three-dimensional (3D) renderings were generated using the M3D module of the MCID Elite software (GE Healthcare, Chalfont St. Giles, UK). Z-stack distance between slices was set at 1 mm. Muscle, fat, and bone tissue compartments were rendered 3D individually and later merged in separate channels using Photoshop 5.5 software to generate three-color 3D visualizations of CT scans.

Serum hormones and OGTT. A separate group of female rats (n = 5 per group) were challenged with an OGTT at the end of 3 wk of overfeeding. OGTT was also conducted in the male offspring at PND120. Rats were fasted for 6 h (from 9:00 AM to 3:00 PM for female rats) or overnight (in the case of offspring) prior to receiving a 2.5 g/kg oral challenge of glucose (0.5 g/ml). Blood (100 μl) from the tail vein was collected in capillary tubes at the beginning of the fast and at 0, 15, 30, 60, 90, and 150 min following the glucose challenge. Serum glucose was measured using glucose oxidase method (Synermed, Westfield, IN). Serum insulin concentrations were assayed using ELISA for rat insulin (Linco Research, St. Charles, MO). We assessed fed and fasted levels of serum leptin, fed levels of NEFA, and triglycerides in lean and obese female rats at the end of 3
wk of overfeeding. Leptin levels were assayed using ELISA (Linco Research). Serum triglycerides and NEFA levels were estimated using commercially available reagents (triglycerides, Synermed, Westfield, IN and NEFA C reagents, WAKO, Richmond, VA).

Adipose histomorphometry. For histomorphometric analyses 3–4 mm pieces of adipose tissue from the retroperitoneal fat depots were fixed in buffered alcoholic formalin for 4 days and embedded in paraffin using routine histological procedures. Sections (6–μm thick) were stained with hematoxylin and eosin. Diameters of adipocytes were measured using a Zeiss Axiosvert microscope (Carl Zieiss, Thornwood, NY) with ZieissVision software (Carl Zieiss). A minimum of 300 cells at random were measured for each slide (n = 4 per group) and percentage of cells in each size range was computed using MS Excel (Microsoft, Redmond, WA).

Statistical analysis. Data are expressed as means ± SE. Associations between the variables, serum leptin, insulin at 15 min postglucose challenge, and %fat mass, respectively, were examined by linear regression. Similarly, a linear regression analysis was carried out between %fat ratio and body weights in the offspring of lean or obese dams at PND120. Statistical differences between lean and obese rats prior to conception or dams during gestation were determined using Student’s t-test. A two-way ANOVA followed by all-pair-wise comparison by the Student-Newman-Keuls method was used to compare the effects of maternal obesity and postweaning HFD. Statistical significance was set at P < 0.05. Statistical analyses were performed using SigmaStat 3.3 software (Systat Software, San Jose, CA). Graphical representation was performed using SigmaPlot version 10.0 for Windows (Systat Software).

RESULTS

Effect of obesity on body composition and metabolic parameters. Body weight gains for female rats fed liquid diets via total enteral nutrition at 187 kcal/kg\(^{3/4}\) per day or 220 kcal/kg\(^{3/4}\) per day are represented in Fig. 1. Overfeeding 15% excess calories to rats resulted in greater weight gains during the 3-wk infusion period. Obese rats at the end of 3 wk were 21% (P < 0.01) heavier compared with lean controls. Consistent with greater weight gain, overfed rats showed higher body adiposity at 3 wk. NMR analyses revealed that obese rats had a 98% greater %fat mass and 15% lower relative lean mass (P < 0.001, Fig. 2, A and B). X-ray CT analyses confirmed NMR data. Two-dimensional whole body radiograms (Fig. 2C) and transverse sections (Fig. 2D) of representative lean and obese rats are depicted. Higher body adiposity following overfeeding was also evident from 3D renderings of collated CT slices (Fig. 2E). Muscle and fat tissues are rendered in red and blue colors, respectively. 3D renderings of obese rats show greater blue and violet (as a result of merging red and blue colors) compared with primarily red deep in lean rats. Quantification of CT analyses revealed that total, visceral, and subcutaneous %fat mass in obese rats was ~462, 423, and 589% of controls, respectively (Fig. 2F, P < 0.001). In addition, CT analyses also showed %fat ratio was increased to ~424% of lean controls (P < 0.001) following overfeeding (Fig. 2G). Conversely, %lean mass was significantly decreased (P < 0.05) in overfed-obese rats. It should be noted that while NMR assesses body composition of the entire animal, we used X-ray CT analyses to specifically assess body composition in the trunk region of the animals, which contributes differences in the %fat mass and the overall magnitude of increased adiposity between the two techniques. Despite these differences, overfeeding resulted in significant elevations in body weights and adiposity.

Serum glucose, insulin, and leptin levels were assessed following 3 wk of overfeeding. Significant hyperinsulinemia was observed in the obese rats, with approximately fivefold higher fasting serum insulin levels (P < 0.05) indicative of obesity-associated insulin resistance (Table 1), which was confirmed by OGTT. Obese rats had severe insulin resistance (Fig. 3A), which was associated with impaired glucose disposition (hyperglycemia despite hyperinsulinemia, Fig. 3B). Linear regression of %fat mass and serum insulin response (at 15 min) showed significant positive correlation (r\(^2\) = 0.74, P < 0.001). Increased adiposity was also associated with progressively higher fasting and fed serum leptin concentrations (Table 1). Serum leptin in the obese rats was ~4.5-fold higher than in the lean rats. Linear regression of fed serum leptin levels and %fat mass revealed significant positive correlation (r\(^2\) = 0.97, P < 0.001). Furthermore, after normalization of serum leptin levels to %fat mass, increased adiposity was associated with higher leptin values (P < 0.01), suggesting that progressively obese rats were secreting higher serum leptin than would be predicted for their increased fat mass, a hallmark of leptin resistance (Table 1). Serum triglyceride and NEFA levels were also significantly elevated (P < 0.05) in obese rats (Table 1).

Gestational weight gains and effect of maternal obesity on offspring growth. Following resumption of infusion of liquid diets on day 8 at 220 kcal/kg\(^{3/4}\) per day, the rate of pregnancy-related weight gain (gestation days 8–21) was nearly the same in both lean and obese dams (Fig. 4). The body composition differences between the groups, however, remained the same after mating as before mating, i.e., obese dams had greater %fat mass than the lean controls throughout gestation (P < 0.01).

Offspring were reared by surrogate dams that were not cannulated and had ad libitum access to AIN-93G pelleted diets. Hence, offspring exposure to obesity was limited to gestation. Birth weights (lean dams, 6.19 ± 0.3 vs. obese dams, 6.44 ± 0.2 g), numbers of pups (lean dams, 11.7 ± 0.8 vs. obese dams, 11.6 ± 0.88), male-to-female ratio (lean dams, 1.1 ± 0.2 vs. obese dams, 1.5 ± 0.3), crown-to-rump distance (lean dams, 1.6 ± 0.05 vs. obese dams, 1.7 ± 0.02 in.), anogenital distance (0.13 ± 0.008 vs. obese dams, 0.15 ± 0.002 in.), and testes weight (0.6 ± 0.1 vs. obese dams, 0.8 ± 0.03 g) were not significantly different between the two groups. The offspring of obese dams weighed significantly more at birth than the offspring of lean dams (P < 0.05 vs. obese dams, 1.5 g). Intergenic distance was significantly shorter in the offspring of obese dams (0.05 ± 0.01 vs. obese dams, 0.10 ± 0.02 in.).

Table 1. Effect of obesity on endocrine and metabolic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean</th>
<th>Obese</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>138 ± 3.3</td>
<td>122 ± 8</td>
<td>113 ± 5.9*</td>
<td>133 ± 7</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.0 ± 0.1</td>
<td>1.7 ± 0.15</td>
<td>4.1 ± 0.7*</td>
<td>11.2 ± 1.06*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.8 ± 0.6</td>
<td>8.09 ± 0.6</td>
<td>29.4 ± 3.3*</td>
<td>36.4 ± 5.1*</td>
</tr>
<tr>
<td>Leptin, ng/ml, normalized to %fat mass</td>
<td>0.26 ± 0.03</td>
<td>0.45 ± 0.03</td>
<td>0.75 ± 0.05*</td>
<td>0.93 ± 0.1*</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>NA</td>
<td>20.2 ± 4.5</td>
<td>200.7 ± 66.0*</td>
<td>NA</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>NA</td>
<td>0.43 ± 0.1</td>
<td>0.98 ± 0.1*</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are means ± SE. Data were collected from female rats fed diets via total enteral nutrition (TEN) at 187 kcal/kg\(^{3/4}\) per day (lean, n = 5) or 220 kcal/kg\(^{3/4}\) per day (obese, n = 5) for 21 days. Insulation of diets was carried out for 23 h/day via computer-controlled syringe pumps. Serum insulin and leptin were estimated using ELISA. Glucose, triglyceride, and nonesterified fatty acids (NEFA) levels were assayed using colorimetric methods. %Fat mass was determined using whole animal NMR. *Significantly different by Student’s t-test (P < 0.05) compared to lean controls. NA, not assessed.
Body weight gains of male offspring from lean or obese dams on either control AIN-93G or HFD from PND21 to PND130 are represented in Fig. 5. As anticipated, consumption of obesogenic HFD postnatally, resulted in increased weight gain among the male offspring of lean dams fed HFD (Fig. 5). While the body weights of offspring from obese dams did not differ from offspring of lean dams fed AIN-93G diets, male offspring of obese dams gained remarkably more (P < 0.005) body weights on a HFD (Fig. 5). Average body weights at PND130 were 803 ± 24 g for the HFD-offspring of obese dams vs. 692 ± 23 g for HFD-offspring of lean dams. These data suggest that exposure to maternal obesity led to programming of increased susceptibility to obesity in the offspring despite normal birth weight.

Increased obesity in the offspring of obese dams and changes in adipose histomorphometry. Body composition analyses (both NMR and CT analyses) demonstrated that increased weight gain in the obese dam offspring was essentially due to increased fat mass. A two-way ANOVA, followed by Student-Neuman-Keuls post hoc analyses for NMR data at PND90 showed a significant effect of maternal obesity (P = 0.032) and of HFD consumption (P < 0.001). However, at PND120, offspring of obese dams on HFD were bigger than the maximum allowable body mass to be assayed by NMR and hence...
data could not be collected. Representative whole body radiograms (Fig. 6A) and transverse slices (Fig. 6B) of offspring from lean or obese dams are presented. Despite no changes in body weights, obese dam offspring on AIN-93G diets had a ~1.6-fold greater %fat ratio compared with offspring of lean dams on the same diet (Fig. 6C, P < 0.05). Furthermore, obese dam offspring on HFD had a 26% greater %fat ratio and ~34, 25, and 60% increase in total, visceral, and subcutaneous %fat mass, respectively, compared with offspring of lean dams fed the same HFD (Fig. 6, C and E, P < 0.05). Correlation analyses of body weights and %fat ratios (obtained from X-ray CT analyses) showed a highly positive association (r² = 0.866, P < 0.001, Fig. 6D). Changes in body composition and increases in body fat in the offspring of obese dams fed a HFD compared with offspring of lean dams is also evident in 3D reconstructed color renderings of CT slices (Fig. 6F). Again, muscle and fat tissues are rendered in red and blue colors, respectively. Postmortem adipose tissue and organ (liver and kidney) weights are represented in Table 2. As anticipated, HF feeding increased (P < 0.05) liver and adipose tissue weights (normalized to body weight). In addition, as observed via NMR and CT analyses, a significant effect of maternal obesity (P < 0.05) was apparent in liver and adipose tissue weights when normalized to body weight.

HF feeding significantly increased serum glucose, triglyceride, NEFA, insulin, and leptin levels (P < 0.05) in both lean and obese offspring (Table 3). Most strikingly, serum insulin and leptin levels increased by 2.2- and 2.3-fold in offspring from obese dams fed control diet compared with the offspring from lean dams fed the same diet. The overall effect of
Table 2. Characteristics of male offspring of lean or obese dams at PND130

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Offspring of Lean Dams</th>
<th>Offspring of Obese Dams</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HFD</td>
<td>Control</td>
</tr>
<tr>
<td>Body weight</td>
<td>607±25</td>
<td>692±23</td>
<td>650±20</td>
</tr>
<tr>
<td>% Liver weight</td>
<td>3.2±0.07</td>
<td>3.4±0.06</td>
<td>3.4±0.06</td>
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<tr>
<td>% Total fat</td>
<td>4.4±0.6</td>
<td>8.2±0.3</td>
<td>5.8±0.33</td>
</tr>
<tr>
<td>% RP fat</td>
<td>2.0±0.3</td>
<td>4.0±0.2</td>
<td>2.8±0.18</td>
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<tr>
<td>% Gonadal fat</td>
<td>1.7±0.25</td>
<td>3.3±0.17</td>
<td>2.1±0.2</td>
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<tr>
<td>% Perirenal fat</td>
<td>0.6±0.1</td>
<td>0.89±0.17</td>
<td>0.8±0.08</td>
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<tr>
<td>% Kidney weight</td>
<td>0.59±0.02</td>
<td>0.55±0.01</td>
<td>0.57±0.01</td>
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Table 3. Serum parameters of male offspring of lean or obese dams at PND130

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Offspring of Lean Dams</th>
<th>Offspring of Obese Dams</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HFD</td>
<td>Control</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>134±5.7</td>
<td>154±7.4</td>
<td>130±2.3</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>581±162</td>
<td>1498±228</td>
<td>504±125</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>1.3±0.16</td>
<td>2.0±0.16</td>
<td>1.3±0.08</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>3.9±1.0</td>
<td>9.4±1.7</td>
<td>8.7±2.8</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>9.5±4.6</td>
<td>36.2±7.7</td>
<td>22.1±3.8</td>
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<tr>
<td>Leptin, ng/ml, normalized to %fat</td>
<td>1.8±0.7</td>
<td>4.1±0.8</td>
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</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>64.1±1.2</td>
<td>98.2±0.9</td>
<td>86.1±12</td>
</tr>
<tr>
<td>Adiponectin, µg/ml, normalized to %fat</td>
<td>2.2±0.9</td>
<td>1.1±0.2</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>29.3±1.6</td>
<td>28.4±3.1</td>
<td>43.5±6.2</td>
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</table>

Data are expressed as means ± SE. Data were obtained at postnatal day (PND)130 from male offspring of lean or obese dams (n = 6 per group). Statistical differences were determined using a 2-way ANOVA to examine the effects of maternal obesity and postweaning HFD, followed by Student-Newman-Keuls post hoc analyses.
general dogma that children born to overweight women are themselves at higher risk of becoming overweight is upheld by several well-controlled studies. However, the underlying reasons remain unclear. It has been especially challenging to delineate the specific contributions of obesity at conception in determining body composition of the offspring in later life through epidemiological studies due to the large number of confounding variables. The present studies were aimed at addressing this very question using a total enteral nutrition-based overfeeding model in the rat. We used total enteral nutrition for its ability to overfeed rats in a controlled manner bypassing the satiety response that limits ad libitum food intake. Using this model, we replicated many of the metabolic and endocrine features of overweight individuals. Importantly, we were able to exclude parental genetic influences, match gestational weight gain, limit the exposure of maternal obesity in utero, and control lactation efficiency, all of which can be difficult confounding variables in studies with human subjects.

Hence, the model described in the present report specifically examines the metabolic obesegenic burden on the offspring by being in an obese intrauterine environment per se.

Gestational and neonatal programming of obesity risk has previously been examined in several animal models, primarily in rodents but also in larger animal models such as the sheep and pig (2, 18, 58). Consumption of low-protein diets or maternal caloric restriction during gestation invariably results in offspring of low birth weights (13, 23, 38, 47, 52, 53). However, susceptibility to obesity of the offspring appears to be dependent on several factors including timing, degree, and nature of macronutrient/caloric restriction; obesegenic nature of the postnatal diet; and the species being studied (rats, mice, guinea pigs or larger animals). Overall, it appears that caloric restriction (70% of ad libitum diet) or protein restriction (8% protein during gestation) in mice (38, 57), 30–50% of ad libitum diet (13, 53) or intrauterine growth retardation due to uterine artery ligation in rats (48), leads to higher body weight.
and/or adiposity in the offspring, especially when challenged with an obesogenic diet. Similarly, studies of maternal feed restriction in the sheep or guinea pig also show increased adiposity in the fetus (24, 50, 58). Certainly, larger animals like the sheep may be more suitable to study late-gestation effects and fetal adipose tissue development compared with rodents.

An important aspect common to all of the above-mentioned studies is the emphasis on the impact of poor fetal nutrition. While undernutrition and protein deficiency are clearly important in many countries, including the United States, a growing problem in obesity is actually overconsumption of calories which is becoming increasingly common in the United States and is being better documented by basic studies (16, 37). Elegant work by Levin and Govek (28), using diet-resistant and diet-induced obesity-susceptible strains of rats, showed that both genetic factors and maternal obesity interact to influence offspring susceptibility to obesity. Furthermore, recent studies by Khan and colleagues (20–22, 51) have shown that the consumption of diets high in lard, from 10 days prior to conception through weaning, lead to increased hypertension, hyperinsulinemia, adiposity, and endothelial dysfunction in the offspring at 6 mo of age. Feeding high-saturated fat diets ad libitum for 10 days prior to conception led to only a modest increase in body weights at conception (21). Rats fed the lard diet showed a dramatic increase in body weight gain during gestation, suggesting that this model may mimic the risk factors associated with increased weight gain during pregnancy rather than prepregnancy body composition (21). Recent studies by Oken et al. (37) suggest that high gestational weight gain significantly increases the risk of childhood obesity. Our data suggests that maternal body composition at conception itself has important implications for offspring adiposity. Gestational weight gains in both the lean and obese rats did not differ, suggesting that offspring adiposity is susceptible to programming during several developmental windows and greater weight gain during gestation may add to the risk.

In the present studies, programming of obesity occurs in the absence of changes in birth weights of offspring and other parameters, such as litter and offspring size as a function of maternal obesity. These findings are consistent with reports demonstrating that HF feeding during gestation does not affect birth weight (51), suggesting subtle programming of obesity may occur in the absence of obvious changes in birth weight (17). On the other hand, Yamashita et al., (55) reported that in a model of GDM using the homozygous C57BL/Ks-Leprdb/db+ mice, increased obesity and insulin resistance in the offspring was associated with higher birth weights, consistent with findings from offspring of GDM mothers (40). Since we did not observe any increase in birth weights, it is likely that maternal obesity in our model is not associated with GDM. Moreover, our data indicate that fetal programming caused by maternal obesity was evident even when fed control AIN-93G diets. However, the effects of maternal obesity were reinforced when challenged with an obesogenic HFD implicating an important role of postnatal diet in modifying adiposity. These data beg the question as to whether children born appropriate for gestational age (in birth weight) to overweight/obese individuals may still be at higher risk of developing obesity under certain postnatal environments. Li et al. (29) suggested that maternal overweight (BMI: 25–29.9) doubled the odds of childhood overweight in a cohort, where GDM and low or high birth weights were excluded. Similarly, Gillman et al. (16) found maternal BMI to be an influencing variable in associations between maternal BMI, GDM, and offspring obesity. Taken together, our data from animal experiments and population-based studies indicate that maternal overweight at conception itself may contribute to offspring obesity risk.

The detailed analyses of body composition in both the dams and offspring is an important strength of the present studies. In addition to quantitatively estimating fat and lean mass, CT analysis allows examination of the distribution of body fat between subcutaneous and visceral fat pads. Our findings suggest that overall adipogenesis is increased in the offspring of obese animals. Consistent with increased adipogenesis, expression of the adipogenic transcription factor PPAR-γ was significantly higher in the adipose tissue. Similar findings were reported by Muhlhauser et al., (34) who also found increased PPAR-γ levels in the fetal perirenal adipose tissue of sheep that were overfed during late gestation. Hence, it appears that regulators of the adipogenic program may be susceptible to fetal programming. Furthermore, histomorphometric analyses of adipose tissues clearly revealed a greater percentage of very large adipocytes that have been shown to be more insulin resistant compared with smaller adipocytes (11). Increased whole body insulin resistance was also confirmed by OGTT, indicating greater overall insulin resistance in the obese dam offspring. Certainly, decreased adiponectin and increased serum resistin as observed in our studies, have been shown to be associated with insulin resistance (49). Although speculative at this point, programming of the offspring hypothalamic-pituitary-adrenal axis, adipose levels of HSD-1, and peripheral leptin signaling are plausible mechanisms. In addition, levels of other pro-/anti-adipogenic factors, such as C/EBP-α, Pref-1, wt-10b, and lipogenic factors, such as SREBP-1 and LPL can be altered by programming. Interestingly, our results suggest that maternal obesity does not appear to alter food intake and that adiposity in the offspring of obese dams occurs in the face of consumption of equal calories. However, since we measured food intake only at two periods in the study, these data may need to be interpreted with caution and more detailed assess-
ment of food intake and energy expenditure via indirect calo-
rimetry are warranted.

In conclusion, we have demonstrated that obesity at concep-
tion in the rat brought about by overfeeding of nutritionally complete diets led to increased obesity in the offspring. Pro-
gramming of obesity occurs in the absence of changes in birth weights and is associated with significant alterations in meta-
bolic and endocrine parameters and adipose tissue cellularity and appears to be independent of caloric intake. Contributions of maternal body composition at conception in increasing offspring susceptibility to obesity may have important impli-
cations in identifying effective strategies of primary preven-
tion.

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REFERENCES

13. Desai M, Gayle D, Babu J, Ross MG. Programmed obesity in intrauter-
18. Gnanalingham MG, Mostyn A, Symonds ME, Stephenson T. Ontog-
24. Kidl KL, Roberts CT, Sohstahl AI, Katsman A, Clifton PM, Rob-
28. Levin BE, Govek E. Gestational obesity accentuates obesity in obesity-
32. Mei Z, Grummer-Strawn LM, Scanlon KS. Does overweight in infancy persist through the preschool years? An analysis of CDC Pediatric Nutri-
34. Muhlhauuser BS, Duffield JA, McMillen IC. Increased maternal nutri-


