Exposure to maternal overnutrition and a high-fat diet during early postnatal development increases susceptibility to renal and metabolic injury later in life

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Jackson CM, Alexander BT, Roach L, Haggerty D, Marbury DC, Hutchens ZM, Flynn ER, Marie-Bilkan C. Exposure to maternal overnutrition and a high-fat diet during early postnatal development increases susceptibility to renal and metabolic injury later in life. Am J Physiol Renal Physiol 302: F774–F783, 2012.—Overnutrition during pre- and postnatal development both confer increased susceptibility to renal and metabolic risks later in life; however, whether they have an additive effect on the severity of renal and metabolic injury remains unknown. The present study tested the hypothesis that a combination of a pre- and postnatal diet high in fat/fructose would exacerbate renal and metabolic injury in male offspring later in life. Male offspring born to high-fat/high-fructose-fed mothers and fed a high-fat/high-fructose diet postnatally (HF-HF) had increased urine albumin excretion (450%), glomerulosclerosis (190%), and tubulointerstitial fibrosis (101%) compared with offspring born to mothers fed a standard diet and fed a standard diet postnatally (NF-NF). No changes in blood pressure or glomerular filtration were observed between any of the treatment groups. The HF-HF offspring weighed ~23% more than offspring born to mothers fed a high-fat/high-fructose diet and fed a normal diet postnatally (HF-NF), as well as offspring born to mothers fed a standard diet regardless of their postnatal diet. The HF-HF rats also had increased (and more variable) blood glucose levels over 12 wk of being fed a high-fat/high-fructose diet. A combination of exposure to a high-fat/high-fructose diet in utero and postnatally increased plasma insulin levels by 140% compared with NF-NF offspring. Our data suggest that the combined exposure to overnutrition during fetal development and early postnatal development potentiate the susceptibility to renal and metabolic disturbances later in life.

Offspring of Wistar rats fed a high-fat diet during early postnatal development display marked increases in adult body weight and insulin resistance over and above that displayed by offspring of rats fed a normal-fat diet during postnatal life (24). Similarly, offspring born to dams fed a cafeteria-style diet during gestation followed by exposure of the offspring to a similar diet during early postnatal development exhibited increased body weight and body mass index at 10 (7) and 12 wk of age (8) compared with offspring fed a standard diet. In addition to exposure to a diet high in fat, a fructose-rich diet during lactation programs the offspring to metabolic-neuroendocrine dysfunction later in life (3). Interestingly, while several studies suggest that exposure to a high-fat diet in adulthood leads to renal injury (2, 13), the delayed effects of early overnutrition on adult renal function have yet to be fully examined.

Considering the global obesity epidemic (11), which affects both the adult and early childhood populations, the importance and necessity of examining the impact of maternal and early childhood overnutrition on long-term health are apparent. Thus the aim of the present study was to test the hypothesis that coupling of high fat/fructose consumption during pregnancy with exposure to a high-fat/high-fructose diet in postnatal life will increase the severity of metabolic and renal dysfunction of offspring in adulthood.

MATERIALS AND METHODS

Experimental design: dams. Female Sprague-Dawley rats (12 wk of age) were purchased from Harlan (Madison, WI) and fed either a standard rat chow diet (29% protein, 55% carbohydrate, and 16% fat), or a diet high in fat (19% protein, 36% carbohydrate, and 45% fat) for 6 wk before mating. All animals were provided tap water ad libitum, but animals fed the high-fat diet were also provided water containing 0.1 g/ml fructose. Before mating, the animals were placed in metabolic cages for determination of urine output and food and caloric intake. The female rats were then bred with normal male Sprague-Dawley rats fed the standard rat chow and tap water. Female rats were maintained throughout pregnancy and lactation on their designated diets of either normal rat chow (n = 8) or high fat/high fructose (n = 6). Throughout gestation, their blood glucose and body weights were measured weekly and blood pressure and glomerular filtration rate (GFR) were measured at the time of euthanasia, which was 1 day after their offspring were weaned, as described below. At the time of euthanasia, visceral fat was dissected out from each animal and weighed.

Experimental design: offspring. Two days following delivery, litters were culled to the same number of offspring to control for equal access to nourishment during lactation. In the process of culling, the ratio of male to female offspring was maintained equal in all the litters. Pups were weaned at 5 wk postnatally. At weaning, all male offspring were randomly divided into two groups, those fed a standard...
ing them 17 wk of age at the time of euthanasia.

Groups were on their respective diets for 12 wk postweaning, render-
and fed a high-fat/high-fructose diet postnatally. Offspring in the four
HF-HF (offspring of mothers fed a high-fat/high-fructose diet and fed a standard diet postnatally;
HF-NF (offspring of mothers fed a high-fat/high-fructose diet and fed a standard diet postnatally;
HF-HF (n = 6), offspring of mothers fed a high-fat/high-fructose diet and fed a high-fat/high-fructose diet postnatally; and HF-NF to offspring of mothers fed a high-fat/high-fructose diet postnatally. Offspring in the four groups were on their respective diets for 12 wk postweaning, render-
ing them 17 wk of age at the time of euthanasia.

Throughout the study, blood glucose levels (in a 10-μl sample obtained from the tail vein) were monitored in the offspring weekly using a FreeStyle Lite glucometer (Abbott Diabetes Care, Alameda, CA). Four days before euthanasia, the offspring were placed in metabolic cages for 24 h for measurement of urine output and various metabolic parameters. The animals were then returned to their regular cage and fasted overnight before undergoing an oral glucose tolerance test. Immediately following the glucose tolerance test, all animals were instrumented with catheters for measurement of blood pressure and renal function as described below. Following an overnight recovery, the measurements were recorded in conscious animals and the kidneys were removed and weighed at the end of the hemodynamic protocol. Parts of the renal cortex were either snap frozen in liquid nitrogen (for protein analysis) or were fixed with 10% buffered formalin (for histology and immunohistochemistry). At the time of euthanasia, visceral fat was dissected out from each animal and weighed.

All experiments were performed according to the guidelines rec-
ommended by the National Institutes of Health and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Measurement of mean arterial pressure and renal function (in
dams and offspring). Animals were anesthetized with 3% isoflurane,
and catheters were placed in the femoral artery for recording of
arterial pressure and in the femoral vein for intravenous infusions and
blood collection. After overnight recovery, mean arterial pressure
(MAP) was continuously recorded for 2 h in conscious rats via a
pressure transducer connected to a computerized data-acquisition
system (PowerLab, ADInstruments, Colorado Springs, CO). The GFR
was measured in conscious, restrained rats by infusing \(^{125}\)Iiothal-
amate (10 μCi/ml 0.9% sodium chloride) at a rate of 2 ml/h over 3 h.
After the 3 h of equilibration, three blood samples (50 μl each) were
taken at 30-min intervals. The GFR was then calculated as radioactive
counts per minute (cpm) for infusate X infusion rate divided by cpm
for plasma samples and expressed as milliliters per minute per gram
kidney weight.

Urine albumin excretion. Urine albumin concentration was mea-
sured in urine collected gravimetrically for 24 h using a Nephriet II
albumin kit (Exocel, Philadelphia, PA) according to the manufacturer’s
protocol and as previously described (32).

Oral glucose tolerance test. After an overnight fast, rats were given
50% glucose in water by oral gavage (1 ml/100 g body wt). Glucose
was measured in tail blood using a glucometer at time 0 and then 15,
30, 60, 120, and 150 min after gavage. The data are presented as area
under the curves.

Glomerulosclerosis and tubulointerstitial fibrosis. To assess mark-
ers of renal pathology, indices of glomerulosclerosis (GSI) and tubu-
lointerstitial fibrosis (TIFI) were evaluated using a semiquantitative
scoring method as previously described by a reviewer blinded to
sample identity (32).

Immunohistochemistry. Paraffin-embedded sections (4 μm) were
incubated with 10% nonimmune goat or 0.1% bovine serum to block
nonspecific immunolabeling. The sections were then incubated with
antisera against nestin (1:200, mouse monoclonal, Millipore, Bil-
gerica, MA). Following washes with phosphate-buffered saline, the

Table 1. Metabolic parameters in dams at the time of euthanasia

<table>
<thead>
<tr>
<th></th>
<th>NF, n = 8</th>
<th>HF, n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>319 ± 6.10</td>
<td>375 ± 8.75†</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>27.0 ± 1.0</td>
<td>22.4 ± 1.5†</td>
</tr>
<tr>
<td>Caloric intake, kcal/day</td>
<td>84.3 ± 5.1</td>
<td>128.0 ± 4.6†</td>
</tr>
<tr>
<td>Visceral fat, g</td>
<td>5.0 ± 0.49</td>
<td>11.8 ± 1.4†</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>116 ± 3.4</td>
<td>129.0 ± 7.2†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>110 ± 8.7</td>
<td>108 ± 8.1</td>
</tr>
</tbody>
</table>
| GFR, ml/min 
\(^{-1}\)·g kidney wt 
\(^{-1}\) | 1.99 ± 0.07 | 1.84 ± 0.06‡|

Values are means ± SE. NF, normal-fat diet; HF, high-fat diet; MAP, mean arterial pressure; GFR, glomerular filtration rate. Statistical significance was accepted at \(P < 0.05. \) *\(P < 0.05, †P < 0.001 vs. NF.

Fig. 1. Effects of maternal overnutrition and postnatal diet on body weight of offspring. A. body weight; B. visceral fat. Values are means ± SE. NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6, where NF-NF refers to
offspring of mothers fed a standard diet and fed a standard diet postnatally; NF-HF to offspring of mothers fed a standard diet and fed a high-fat/high-fructose diet postnatally; HF-NF to offspring of mothers fed a high-fat/high-fructose diet and fed a standard diet postnatally; and HF-HF to offspring of mothers fed a high-fat/high-fructose diet postnatally.
Table 2. *Metabolic and renal parameters in offspring at 17 wk of age*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NF-NF, n = 10</th>
<th>HF-NF, n = 6</th>
<th>NF-HF, n = 10</th>
<th>HF-HF, n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g</td>
<td>29.7 ± 1.92</td>
<td>33.8 ± 1.17</td>
<td>21.6 ± 0.77†</td>
<td>20.5 ± 1.4†</td>
</tr>
<tr>
<td>Caloric intake, kcal/day</td>
<td>88.4 ± 19.8</td>
<td>101.4 ± 9.8</td>
<td>96.8 ± 11.2</td>
<td>102.4 ± 4.1</td>
</tr>
<tr>
<td>Kidney/body weight, mg/g</td>
<td>6.4 ± 0.2</td>
<td>6.6 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>125.0 ± 2.6</td>
<td>125.0 ± 2.6</td>
<td>129.0 ± 2.4</td>
<td>131.0 ± 2.7</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g kidney wt⁻¹</td>
<td>1.84 ± 0.3</td>
<td>1.42 ± 0.3</td>
<td>1.81 ± 0.2</td>
<td>1.67 ± 0.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. NF-NF, offspring of mothers fed a standard diet and fed a standard diet postnatally; HF-NF, offspring of mothers fed a high-fat/high-fructose diet and fed a normal diet postnatally; NF-HF, offspring of mothers fed a standard diet and fed a high-fat/high-fructose diet postnatally; HF-HF, offspring of mothers fed a high-fat/high-fructose diet and fed a high-fat/high-fructose diet postnatally. Statistical significance was accepted at \( P < 0.05 \). *\( P < 0.05 \) vs. NF-F. †\( P < 0.05 \) vs. HF-NF.

Fig. 2. Effects of maternal overnutrition and postnatal diet on blood glucose and plasma insulin. A: blood glucose. B: glucose tolerance test including area under the curve in arbitrary units (AUC in AU). C: plasma insulin. Values are means ± SE; NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6.

F776 OBESITY-ASSOCIATED KIDNEY AND METABOLIC INJURY IN OFFSPRING

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sections were incubated with the appropriate secondary, biotinylated IgGs and finally with the avidin-biotin complex (Vectastain ABC kit, Elite, Vector Laboratories). A positive immunoreaction was identified following a 10-min treatment with 3,3-diaminobenzidine and counterstaining with Mayer’s hematoxylin.

Following staining, 30 glomeruli were randomly selected and the density of nestin-positive cells was quantified using image-analysis software (NISS-Elements, Ver. 2.32; Nikon Instruments, Melville, NY).

Western blotting. Homogenized denatured protein samples were separated through SDS-PAGE precast gels (Bio-Rad, Hercules, CA) and then transferred to a nitrocellulose membrane. The membranes were then blocked first with 5% nonfat milk and then incubated overnight in 4°C with antisera against transforming growth factor (TGF)-β (1:500 rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA) and podocin (1:2,000 rabbit polyclonal, Abcam, Cambridge, MA). Then, the membranes were incubated with the appropriate secondary antibodies conjugated to horseradish peroxidase, and proteins were visualized by enhanced chemiluminescence (Thermo Scientific, Rockford, IL). All the membranes were stripped using a stripping buffer (Thermo Scientific) and reprobed with an antibody against β-actin (1:1,000 mouse monoclonal, Cell Signaling, Danvers, MA). The densities of the specific bands were quantitated using Scion Image beta (version 4.02) software and then normalized to the amount of protein loaded in each well using the densitometric analysis of Image beta (version 4.02) software and then normalized to the amount of protein loaded in each well using the densitometric analysis of Image beta (version 4.02) software.

Statistical analysis. All values are expressed as means ± SE and were analyzed using one-way ANOVA (Prism 4, Graph Pad Software, San Diego, CA). Post hoc comparisons were performed using the Newman-Keuls multiple comparison test. Differences were considered statistically significant at P < 0.05.

RESULTS

Maternal metabolic data. At the time of euthanasia (i.e., immediately after weaning), HF dams weighed 18% more than NF dams and had 136% more visceral fat than NF dams (Table 1). This was accompanied by an average of 20% increase in food intake and a 52% increase in caloric intake over the treatment period in HF compared with NF dams (Table 1). No differences in blood glucose, MAP, or GFR were observed between NF and HF dams at the time of euthanasia (Table 1). In addition, no differences in litter size or the male/female ratio of the offspring were observed between NF and HF dams (Table 1). Maternal metabolic data. At the time of euthanasia (i.e., immediately after weaning), HF dams weighed 18% more than NF dams and had 136% more visceral fat than NF dams (Table 1). This was accompanied by an average of 20% increase in food intake and a 52% increase in caloric intake over the treatment period in HF compared with NF dams (Table 1). 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Blood glucose levels were statistically similar throughout the study in all treatment groups (Fig. 2 A). However, from approximately postnatal day 63 (i.e., 4 wk of a high-fat/high-fructose postnatal diet), there was a tendency toward slightly elevated and more variable glucose levels in offspring that were fed a high-fat diet, regardless of the maternal nutritional status (Fig. 2 A). Oral glucose tolerance tests revealed no statistically significant differences among groups (Fig. 2 B). Both the maternal nutritional status and postnatal diet had significant effects on plasma insulin levels in the offspring at the time of euthanasia (Fig. 2 C). HF-NF animals had 42% higher plasma insulin levels than NF-NF, indicating that maternal overnutrition alone is associated with a metabolic phenotype in the offspring born to mothers also fed the same fat/fructose-rich diet. No differences in kidney/body weight were observed among any of the treatment groups (Table 2).

Offspring fed a normal fat diet consumed ~34% more food than offspring fed a high-fat/high-fructose diet (Table 2). However, once food intake was corrected for caloric value per gram of consumed food and fluid, no differences in caloric intake were observed among any of the treatment groups (Table 2), suggesting that differences in any parameters measured in the study are not likely to be due to differences in calories consumed.

Despite similar daily caloric intake between groups, offspring fed a high-fat/high-fructose diet postnatally had increased amounts of visceral fat (Fig. 1 B). Specifically, NF-HF offspring had 93% more visceral fat than NF-NF, while HF-HF offspring had 99% more visceral fat than HF-NF (Fig. 1 B). Furthermore, HF-HF offspring had 30% more visceral fat than NF-HF, suggesting that maternal overnutrition potentiates visceral fat accumulation after exposure to a high-fat/high-fructose diet postnatally.

Effects of maternal overnutrition and high-fat postnatal diet on glucose tolerance and plasma insulin. Blood glucose levels were statistically similar throughout the study in all treatment groups (Fig. 2 A). However, from approximately postnatal day 63 (i.e., 4 wk of a high-fat/high-fructose postnatal diet), there was a tendency toward slightly elevated and more variable glucose levels in offspring that were fed a high-fat diet, regardless of the maternal nutritional status (Fig. 2 A). Oral glucose tolerance tests revealed no statistically significant differences among groups (Fig. 2 B).

Both the maternal nutritional status and postnatal diet had significant effects on plasma insulin levels in the offspring at the time of euthanasia (Fig. 2 C). HF-NF animals had 42% higher plasma insulin levels than NF-NF, indicating that maternal overnutrition alone is associated with a metabolic phenotype in the offspring born to mothers also fed the same fat/fructose-rich diet. No differences in kidney/body weight were observed among any of the treatment groups (Table 2).

Fig. 3. Effects of maternal overnutrition and high-fat postnatal diet on urine albumin excretion (UAE). Values are means ± SE; NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6.
offspring. In addition, insulin levels in NF-HF animals were 87% higher than in NF-NF, suggesting that a high-fat/high-fructose postnatal diet alone results in a metabolic phenotype in the offspring. Because HF-HF animals had insulin levels that were 140% higher than those in the NF-NF group suggests that maternal nutritional status and postnatal diet may act synergistically on metabolic control in the offspring.

Effects of maternal overnutrition and high-fat postnatal diet on renal function. Albuminuria varied according to the nutritional status of the mother in that HF-NF animals had a 209% higher UAE than NF-NF (Fig. 3). In addition, postnatal diet also influenced the development of albuminuria as evidenced by an 83% increase in UAE in NF-HF rats compared with NF-NF and a 78% increase in HF-HF compared with HF-NF rats (Fig. 3). Because the HF-HF group had 450% greater UAE compared with NF-NF suggests that maternal nutritional status and postnatal diet may act synergistically on UAE.

Neither maternal overnutrition nor a high-fat postnatal diet appeared to have an impact on MAP or GFR, as there were no significant differences between groups on these values (Table 2).

Effects of maternal overnutrition and high-fat postnatal diet on renal pathology. Glomerulosclerosis was defined as an expansion of mesangial areas and dilatation of intraglomerular capillaries. As pictured in Fig. 4A, maternal overnutrition was associated with glomerulosclerosis in the offspring regardless of their postnatal diet. Indeed, HF-NF animals had a 64% increase in GSI compared with NF-NF, while HF-HF rats had a 190% increase in GSI compared with NF-HF (Fig. 4B). Furthermore, GSI was increased by 88% in HF-HF compared with HF-NF rats. These observations suggest that a high-fat

Fig. 4. Effects of maternal overnutrition and high fat postnatal diet on glomerular injury. A: periodic acid-Schiff-stained sections. B: index of glomerulosclerosis (GSI) expressed in AU. Values are means ± SE; NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6.
postnatal diet may exacerbate the effects of maternal overnutrition on renal pathology (Fig. 4B).

Tubulointerstitial fibrosis was defined as an accumulation of extracellular matrix, tubular dilatation or atrophy, and the presence of inflammatory cells and tubular casts. As pictured in Fig. 5A, a high-fat diet postnatally was associated with increased fibrosis regardless of maternal nutritional status. Specifically, NF-HF was associated with a 59% increase in TIFI compared with NF-NF, and HF-HF was associated with a 56% increase in TIFI compared with HF-NF. Maternal overnutrition did not appear to exacerbate the effects of the high-fat/high-fructose postnatal diet (Fig. 5B).

Effects of maternal overnutrition and high-fat postnatal diet on renal TGF-β protein expression and podocyte injury. As indicated in Fig. 6, there were no differences in renal cortical TGF-β protein expression among NF-NF, NF-HF, and HF-NF offspring; however, HF-HF rats displayed higher levels of TGF-β protein expression than all other groups, suggesting that only the combined effects of maternal overnutrition and a high-fat/high-fructose diet postnatally affect TGF-β protein expression.

Podocyte injury was determined by measuring protein expression of podocin, which is an integral protein component of the glomerular filtration barrier (26). As indicated in Fig. 7A, a high-fat/high-fructose postnatal diet was associated with decreased expression of podocin, suggesting podocyte injury, irrespective of maternal nutritional status. Image analysis of nestin immunoexpression (Fig. 7B), another integral component of the slit diaphragm (30), also showed that the high-fat/high-fructose postnatal diet contributed to podocyte injury irrespective of maternal nutritional status. In addition, the further decrease in nestin immunoexpression in the HF-HF offspring.
The severity of both renal and metabolic dysfunction. Overnutrition in intrauterine and early postnatal life increases metabolic health in later life and that the combined effects of both in utero and postnatally have a profound effect on renal and metabolic health. These results confirm our hypothesis that early overnutrition greatly increased body weight, and variable glucose levels.

Cortical TGF-β expression and increased podocyte injury, more severe hyperinsulinemia, albuminuria, and glomerulosclerosis. The offspring of mothers fed a high-fat/high-fructose diet developed hyperinsulinemia, albuminuria, and glomerulosclerosis. If the offspring of mothers fed a high-fat/high-fructose diet postnatally (NF-HF) were predisposed toward developing increased visceral fat, more variable glucose levels, hyperinsulinemia, tubulointerstitial fibrosis, and podocyte injury later in life. Offspring born to mothers fed a high-fat/high-fructose diet during gestation and fed a high-fat/high-fructose diet postnatally (NF-HF) were predisposed toward developing increased visceral fat, more variable glucose levels, hyperinsulinemia, tubulointerstitial fibrosis, and podocyte injury later in life. Offspring born to mothers fed a high-fat/high-fructose diet during gestation and fed a high-fat/high-fructose diet postnatally (NF-HF) were predisposed toward developing increased visceral fat, more variable glucose levels, hyperinsulinemia, tubulointerstitial fibrosis, and podocyte injury later in life. These results confirm our hypothesis that early overnutrition both in utero and postnatally has a profound effect on renal and metabolic health in later life and that the combined effects of overnutrition in intrauterine and early postnatal life increase the severity of both renal and metabolic dysfunction.

DISCUSSION

The present study examined the independent and combined effects of exposure to maternal overnutrition (i.e., high fat/high-fructose consumption during gestation) and a high-fat/high-fructose diet during early postnatal development on renal and metabolic function in the offspring later in their lives. The data indicate that offspring born to mothers fed a standard diet during gestation and fed a high-fat/high-fructose diet postnatally (NF-HF) were predisposed toward developing increased visceral fat, more variable glucose levels, hyperinsulinemia, tubulointerstitial fibrosis, and podocyte injury later in life. Offspring born to mothers fed a high-fat/high-fructose diet during gestation and fed a standard diet postnatally (HF-NF) developed hyperinsulinemia, albuminuria, and glomerulosclerosis. If the offspring of mothers fed a high-fat/high-fructose diet during gestation were also fed the same high-fat/high-fructose diet in early postnatal life (HF-HF), they developed more severe hyperinsulinemia, albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis, in addition to elevated renal cortical TGF-β expression and increased podocyte injury, greatly increased body weight, and variable glucose levels. These results confirm our hypothesis that early overnutrition both in utero and postnatally has a profound effect on renal and metabolic health in later life and that the combined effects of overnutrition in intrauterine and early postnatal life increase the severity of both renal and metabolic dysfunction.

The majority of studies examining fetal programming of renal and metabolic disease in adulthood focus on models of fetal undernutrition (e.g., low-protein diet during pregnancy or placental insufficiency) (1, 6, 31). These studies suggest that undernourished animals and babies may be born with a reduced number of nephrons, which, in turn, may lead to the development of hypertension and chronic kidney disease later in life (16, 31). Similarly, children born to undernourished mothers have a reduced number and function of pancreatic β-cells as well as insulin resistance (5, 12). The importance of the early postnatal environment on long-term metabolic, and to a lesser extent renal, health has also been recognized (22, 27). Interestingly though, much less is known about the impact of maternal overnutrition (such as exposure to a high-fat/high-fructose diet during gestation) in combination with an early postnatal diet high in fat/fructose on metabolic and renal injury later in life. This is somewhat surprising given the obesity epidemic that affects both adults and children (11). As indicated above, the present study shows that both maternal obesity and early postnatal exposure to a high-fat/high-fructose diet exacerbate albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis. These changes were associated with increased expression of TGF-β and decreased podocin and nestin expression, suggesting that activation of the proinflammatory pathways and podocyte injury could contribute to the observed renal structural changes and albuminuria, respectively. Previous studies demonstrate that exposure to a high-fat diet in early postnatal life is associated with renal lipid accumulation (14), which may, in turn, lead to activation of proinflammatory pathways and downstream renal injury. The fact that we found no evidence of renal lipid accumulation following either exposure to high fat/high fructose during pre-or early postnatal life (data not shown) suggests the proinflammatory pathways activated are not triggered by lipids accumulated in the kidney. Further studies are warranted to precisely examine the mechanisms underlying inflammation and renal injury in this model.

As mentioned above, the developmental programming of kidney structure and function has thus far mostly been studied in rats undernourished in utero. Intraterine growth restriction as a result of placental insufficiency in the rat is associated with a decreased number of glomeruli and altered renal function, as noted by decreased GFR and renal plasma flow (1, 21, 31). Other studies in offspring of Sprague-Dawley rats undernourished during intrauterine development noted increased systolic blood pressure and increased renin activity compared with offspring exposed to normal, healthy nutrition during intrauterine development (6, 31). In contrast, the present study, which examined the effects of maternal and postnatal overnutrition, did not observe changes in blood pressure or GFR in the male offspring, at least after 17 wk of life. These observations suggest that albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis observed in response to a high-fat/high-fructose insult were independent of changes in renal hemodynamics. The most likely explanation for the renal injury would be a direct effect of proinflammatory cytokines on the renal parenchyma. It is possible, however, that changes in MAP and GFR may develop with aging, which would further contribute to the progression of renal injury. Future studies need to address whether indeed more severe injury develops with aging in the offspring exposed to a high-fat/high-fructose diet during pre- or postnatal life.

Fig. 6. Effects of maternal overnutrition and high-fat postnatal diet on renal cortical transforming growth factor (TGF)-β protein expression. Top: representative immunoblot of TGF-β protein expression. Bottom: densitometric scans in relative optical density (ROD) expressed as the TGF-β/β-actin ratio. Values are means ± SE; NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6.
Supporting findings from previous studies, the present study indicates that exposure to a high-fat diet during prenatal and postnatal life both independently influence visceral fat and plasma levels of insulin (7, 17, 24). The present study also suggests that the combination of the prenatal and postnatal environment has an additive effect on visceral fat, plasma insulin levels, and, in particular, body weight. Indeed, increased body weight was only observed in the male animals that were exposed to high fat/high fructose during pre- and postnatal life. Interestingly, this increase in body weight only became apparent after 9 wk of age, coinciding with the onset of blood glucose variability. While glucose variability has been linked to increased risk of cardiovascular disease (9), further studies are needed to determine whether it can also contribute to long-term weight gain. Furthermore, the blood glucose variability and weight gain also coincided with the

Fig. 7. Effects of maternal overnutrition and high-fat postnatal diet on renal cortical nestin and podocin protein expression. A: podocin protein expression. Top: representative immunoblot of podocin protein expression. Bottom: densitometric scans in ROD expressed as the podocin/β-actin ratio. B: nestin immunolocalization (brown staining, left). Original magnification ×400 and image analysis (right). Values are means ± SE; NF-NF, n = 10; NF-HF, n = 10; HF-NF, n = 6; HF-HF, n = 6.
onset of puberty in these animals, suggesting that testosterone may contribute to weight gain and glucose variability as well as potentially renal injury itself. Future studies will be designed to test this hypothesis in our model.

Several, albeit not all, studies have suggested that exposure to pre- and postnatal overnutrition has a greater impact on renal and metabolic function in female offspring compared with male offspring (10, 19). This is somewhat surprising given that the incidence and prevalence of renal disease in men is significantly greater in men compared with women (28). Given this fact of greater prevalence of renal disease in men, the present study focused only on the male offspring. While further studies are needed to determine whether female offspring exhibit similar changes in metabolic and renal health in our experimental model, the data shown clearly indicates that male offspring are certainly susceptible to metabolic and renal injury following exposure to overnutrition both in utero and postnatally.

In summary, maternal overnutrition is associated with hyperinsulinemia, albuminuria, and glomerulosclerosis in male offspring later in life. A high-fat/high-glucose postnatal diet is associated with increased amounts of visceral fat, more variable glucose levels, hyperinsulinemia, albuminuria, and tubulointerstitial fibrosis later in life of the offspring. Most importantly, the coupling of maternal overnutrition with a high-fat/high-glucose postnatal diet was associated with hyperinsulinemia, albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis to an even greater extent, in addition to greatly increased body weight and variable insulin levels. These results confirm our hypothesis that coupling of high-fat/high-glucose consumption during pregnancy with exposure to a high-fat/high-glucose diet in postnatal life increases the severity of renal and metabolic dysfunction of offspring in adulthood. Finally, these data have direct implications in humans, in which overnutrition during early life and adulthood, leading to obesity, increases the risk of renal and metabolic diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.M.J. and C.-M.B. analyzed data; C.M.J. and C.M.-B. interpreted results of experiments; C.M.J. drafted manuscript; B.T.A. and C.M.-B. edited and revised manuscript; L.R., D.H., D.C.M., Z.M.H., and C.M.-B. provided conception and design of research; C.M.-B. prepared figures; C.M.-B. approved final version of manuscript; L.R., D.H., D.C.M., Z.M.H., and C.M.-B. interpreted results of experiments; C.M.J. drafted manuscript; B.T.A. edited and revised manuscript; L.R., D.H., D.C.M., Z.M.H., and C.M.-B. developed experimental model; E.R.F. performed experiments; C.M.-B. provided conception and design of experimental model, the data shown clearly indicates that male offspring are certainly susceptible to metabolic and renal injury following exposure to overnutrition both in utero and postnatally.

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