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High-fat/fructose feeding during prenatal and postnatal development in female rats increases susceptibility to renal and metabolic injury later in life

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Flynn ER, Alexander BT, Lee J, Hutchens Jr ZM, Maric-Bilkan C. High-fat/fructose feeding during prenatal and postnatal development in female rats increases susceptibility to renal and metabolic injury later in life. Am J Physiol Regul Integr Comp Physiol 304: R278–R285, 2013. First published December 19, 2012; doi:10.1152/ajpregu.00433.2012.—Accumulating evidence suggests that both an adverse prenatal and early postnatal environment increase susceptibility to renal and metabolic dysfunction later in life; however, whether exposure to adverse conditions during both prenatal and postnatal development act synergistically to potentiate the severity of renal and metabolic injury remains unknown. Sprague-Dawley rats were fed either a standard diet or a diet high in fat/fructose throughout pregnancy and lactation. After being weaned, female offspring were randomized to either standard diet or the high-fat/high-fructose diet, resulting in the following treatment groups: NF-NF, offspring of mothers fed a standard diet and fed a standard diet postnatally; NF-HF, offspring of mothers fed a standard diet and fed a high-fat/fructose diet postnatally; HF-NF, offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally; HF-HF, offspring of mothers fed a high-fat/fructose diet and fed a high-fat/fructose diet postnatally. At the time of euthanasia (17 wk of age), HF-HF offspring weighed 30% more and had 110% more visceral fat than NF-NF offspring. The HF-HF offspring also had elevated blood glucose levels, glucose intolerance, 286% increase in urine albumin excretion, and 60% increase in glomerulosclerosis compared with NF-NF. In addition, HF-HF offspring exhibited a 100% increase in transforming growth factor-β protein expression and 116% increase in the abundance of infiltrated macrophages compared with the NF-NF offspring. These observations suggest that high-fat/fructose feeding during prenatal and throughout postnatal life increases the susceptibility to renal and metabolic injury later in life.

MATERNAL MALNUTRITION during pregnancy, defined as either nutrient deficiency or overnutrition, has been shown to adversely affect the health of the offspring later in their life (10, 16, 18). Specifically, maternal obesity or consumption of foods with a high fat content during pregnancy leads to the development of elements of the metabolic syndrome and renal disease in adult offspring (2, 4, 6, 9, 20, 25). Considering that around 50% of women of childbearing age are either overweight (BMI 25–29.9 kg/m2) or obese (BMI ≥ 30 kg/m2) (28), the importance of examining the impact of maternal overnutrition on long-term health of the offspring is apparent.

In addition to the adverse intrauterine environment, accumulating evidence suggests that the conditions of postnatal development, especially in early stages, are also important determinants of long-term susceptibility to chronic diseases in adulthood (9, 19). Specifically related to obesity and overnutrition, studies have shown that offspring fed a high-fat or a fructose-rich diet during early postnatal development are characterized by increased body weight and insulin resistance and have an increased risk of developing metabolic-neuroendocrine dysfunction later in life (1, 20). Interestingly, while there is ample evidence to suggest that obesity and overnutrition increase the risk of chronic kidney disease (12, 13), practically nothing is known about the impact of maternal or postnatal exposure to overnutrition on renal function in the offspring.

While there is strong evidence for either the prenatal or postnatal environment playing an important role in determining susceptibility to chronic diseases later in life, much less is known whether exposure to an adverse environment during both prenatal and postnatal development increases the severity of disease. Studies investigating the combined impact of pre- and postnatal overnutrition have shown that offspring born to dams fed a cafeteria-style diet during gestation followed by exposure of the offspring to a similar diet during postnatal development exhibited increased body weight and body mass index at 10 wk (4) and 12 wk of age (6) compared with offspring fed a standard diet. However, the impact of postnatal overnutrition on adult renal function has not been fully examined. A study from our laboratory has recently shown that combined exposure to a high-fat/fructose diet during fetal and 17 wk of postnatal development potentiate the susceptibility of renal and metabolic disturbances later in life of the male offspring (14).

Given that the incidence and prevalence of renal disease is far greater in males than females (24), it is not at all surprising that the majority of studies examining the impact of pre- and postnatal overnutrition on long-term renal health of the offspring have focused on males. However, accumulating data showing that exposure to pre- and postnatal overnutrition may have a greater impact on metabolic function in female offspring compared with male offspring (8, 30). Thus the aim of the present study was to test the hypothesis that high-fat/fructose feeding during pregnancy combined with high-fat/fructose feeding during postnatal develop-
Development increases the severity of metabolic and renal dysfunction in female offspring later in life.

MATERIALS AND METHODS

Experimental Design

Dams. Twelve-week-old female Sprague-Dawley rats (Harlan, Madison, WI) were fed either a standard rat chow diet (Harlan, cat. no. 8640; protein 29%, carbohydrate 55% and fat 16%; total energy value 3.1 kcal/g), or a diet high in fat (Harlan, cat. no. TD.06415; protein 19%, carbohydrate 36% and fat 45%; total energy value 4.6 kcal/g) and given tap water ad libitum for 6 wk before mating. During these 6 wk, animals randomized to the high-fat diet were also given water containing 10% fructose. The purpose of the fructose supplementation was to increase the overall energy intake. Two days before mating, the animals were placed in metabolic cages for determination of urine output and food and energy intake. These female rats were then mated overnight with male Sprague-Dawley rats fed the standard rat chow and tap water. Throughout pregnancy and lactation, dams were maintained on their designated diets of either normal rat chow diet (n = 8) or the high-fat/high-fructose diet (n = 6).

Offspring. Forty-eight hours after delivery, offspring in all litters were culled to 6 pups (4 males and 2 females) per dam to control for equal access to nourishment during lactation. Pups were weaned at 5 wk postnatally, after which females were randomized to either the standard rat chow diet or the high-fat/high-fructose diet, resulting in the following treatment groups: NF-NF (n = 8), offspring of mothers fed a standard diet and fed a standard diet postnatally; NF-HF (n = 8), offspring of mothers fed a standard diet and fed a high-fat/fructose diet postnatally; HF-NF (n = 6), offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally; HF-HF (n = 6), offspring of mothers fed a high-fat/fructose diet and fed a high-fat/fructose diet postnatally. The offspring were fed their respective diets for 12 wk after weaning, making them 17 wk of age at the time of being euthanized. This time point was chosen based on our previous study in the male offspring (14).

For the duration of the study, the offspring were weighed and their blood glucose levels from tail vein blood samples (Freestyle Lite glucometer, Abbott Diabetes Care, Alameda, CA) measured weekly. Four days before euthanasia, the offspring were placed in metabolic cages for 24 h for urine collection and measurement of food consumption. The animals were then fasted overnight before undergoing an oral glucose tolerance test, following which they were instrumented with catheters for measurement of blood pressure, heart rate (HR), and glomerular filtration rate (GFR). After an overnight recovery, systolic (SBP) and diastolic blood pressure (DBP), HR, and GFR were measured in conscious animals as previously described in detail (14). Mean arterial pressure (MAP) was calculated from SBP and DBP. After these measurements were taken, the rats were anesthetized with isoflurane and the visceral fat was dissected out and weighed. Plasma insulin levels were measured from blood taken from the abdominal aorta at the time of euthanasia using an ELISA assay (Crystal Chem; cat. no. 90060; Downers Grove, IL).

The kidneys were also removed and weighed and then either fixed with 10% buffered formalin (for histology and immunohistochemistry) or frozen in liquid nitrogen (for protein analysis). All experiments were performed according to the guidelines recommended by the National Institutes of Health and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Urine Albumin Excretion

Urine albumin concentration was measured using the Nephrat II albumin kit (Exocel, Philadelphia PA) according to the manufacturer’s protocol and as previously described (32).

Oral Glucose Tolerance Test

Rats undergoing oral glucose tolerance test were fasted overnight (from 4 PM to 8 AM). Immediately before the feeding of glucose, baseline glucose levels (at 0 min) were measured in a blood sample taken from the tail vein. Animals were then orally gavaged with 2 g/kg body weight of glucose in 1 ml of saline and fed ad libitum for 120 min. Glucose levels were measured at 5, 15, 30, 60, and 120 min after glucose feeding using a glucometer (FreeStyle Lite, Abbott Diabetes Care, Alameda, CA) measured weekly.
taken from the tail vein. Rats were then given 50% glucose in water by oral gavage (1 ml/100 g body wt). Glucose was measured in conscious rats in tail blood using a glucometer at time 15, 30, 60, 120, and 150 min following oral gavage.

**Glomerulosclerosis and Tubulointerstitial Fibrosis**

Indices of glomerulosclerosis (GSi) and tubulointerstitial fibrosis (TIFI) were evaluated by a reviewer blinded to sample identity using a semiquantitative scoring method as previously described (32).

**Immunohistochemistry**

Immunolocalization was performed on paraffin-embedded sections as previously described in detail (14) using the following antibodies: nestin (1:200, mouse monoclonal, Millipore, Billerica, MA), and CD68 (1:200, mouse monoclonal, Serotec, Oxford, UK). The density of nestin immunoreactivity was assessed in 30 randomly selected glomeruli per animal using image analysis software (NI-Elements, Ver. 2.32; Nikon Instruments, Melville, NY). The data are expressed as percentage of area stained for nestin per glomerulus. The density of CD-68-positive cells was quantified as we previously described (32). Briefly, CD68-positive cells in 40 different fields per animal from each group were counted and expressed per millimeter squared.

**Western Blotting**

Expression of renal cortical TGF-β (1:500 rabbit polyclonal, Cruz Biotech, Santa Cruz, CA) was performed as previously described (14). The same membrane was stripped 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 total protein loaded in each well using the densitometric analysis of β-actin from the same membrane.

**Statistical Analysis**

All values, except time courses, are expressed as means ± SE and were analyzed using a two-way ANOVA (Prism 4, Graph Pad Software, San Diego, CA). The time course in body weight change and GTF were analyzed using a two-way ANOVA with repeated measures. Post hoc comparisons were performed using the Bonferroni posttest. Differences were considered statistically significant at P < 0.05.

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**Table 1. Metabolic and renal parameters in offspring at 17 wk of age**

<table>
<thead>
<tr>
<th>n</th>
<th>NF-NF</th>
<th>HF-NF</th>
<th>NF-HF</th>
<th>HF-HF</th>
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<tr>
<td></td>
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<tr>
<td>n</td>
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<td>6</td>
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<tr>
<td>Food intake, g/day</td>
<td>25.3 ± 2.6</td>
<td>33.5 ± 4.0</td>
<td>14.1 ± 0.56</td>
<td>17.0 ± 1.5</td>
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<td>Food intake/body wt, g/day·kg⁻¹</td>
<td>102.0 ± 10.3</td>
<td>123.8 ± 9.9</td>
<td>50.2 ± 3.2</td>
<td>53.8 ± 3.7</td>
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<td>Water intake, ml/day</td>
<td>27.9 ± 4.1</td>
<td>25.5 ± 3.4</td>
<td>29.6 ± 10.2</td>
<td>32.3 ± 15.3</td>
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<tr>
<td>Energy intake, kcal/day</td>
<td>78.4 ± 8.0</td>
<td>104.0 ± 12.5</td>
<td>79.3 ± 4.8</td>
<td>93.7 ± 3.4</td>
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<tr>
<td>Energy intake/body wt, kcal/day·kg⁻¹</td>
<td>316.0 ± 31.8</td>
<td>383.9 ± 30.6</td>
<td>280.3 ± 18.2</td>
<td>297.6 ± 8.1</td>
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<td>Kidney/body wt, mg/g</td>
<td>6.38 ± 0.13</td>
<td>6.78 ± 0.10</td>
<td>5.78 ± 0.16</td>
<td>5.75 ± 0.24</td>
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<td>GFR, ml/min·1·g kidney wt⁻¹</td>
<td>2.03 ± 0.050</td>
<td>1.79 ± 0.0056</td>
<td>2.01 ± 0.14</td>
<td>1.89 ± 0.029</td>
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<td>MAP, mmHg</td>
<td>109 ± 4</td>
<td>106 ± 6</td>
<td>112 ± 3</td>
<td>117 ± 4</td>
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<td>SBP, mmHg</td>
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<tr>
<td>HR, mmHg</td>
<td>412 ± 32</td>
<td>417 ± 35</td>
<td>415 ± 18</td>
<td>403 ± 37</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats. GFR, glomerular filtration rate; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NF-NF, offspring of mothers fed a standard diet and fed a standard diet postnatally; HF-HF, offspring of mothers fed a high-fat/fructose diet and fed a high-fat/fructose diet postnatally; NF-NF, offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally; NF-HF, offspring of mothers fed a high-fat/fructose diet and fed a high-fat/fructose diet postnatally. Statistical significance was accepted at P < 0.05. *P < 0.05, **P < 0.01, ***P < 0.001 vs. NF-NF; †P < 0.05, ‡P < 0.01, §P < 0.001 vs. NF-HF; ††P < 0.05 vs. NF-HF.

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**RESULTS**

**Maternal Metabolic Data**

The effect of high-fat/fructose feeding on maternal body weight, energy intake, blood glucose, and renal function has recently been reported (14). Briefly, dams fed a HF diet were characterized by increased body weight and visceral fat but had no changes in blood glucose or renal function (14).

**Effects of High-Fat/Fructose Feeding On Body Weight, Visceral Fat, and Food Consumption**

For the first 7 wk of life, no differences in body weight were observed between any of the experimental groups (Fig. 1A). However, as of postnatal day 56 (i.e., 3 wk of high-fat/fructose feeding) onward, the offspring of mothers fed a high-fat/fructose diet and fed the same diet postnatally (HF-HF) weighed an average of 17% more than all other offspring (P < 0.001; Fig. 1A). This gap in body weight continued to widen through day 119 (i.e., time of euthanasia), at which point HF-HF offspring weighed 23% more than offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally (P < 0.05; HF-NF) and 30% more than offspring of mothers fed a standard diet and fed a standard diet postnatally (P < 0.05; NF-NF). As of postnatal day 77 onward, the body weights of offspring of mothers fed a standard diet and fed a high-fat/fructose diet postnatally (NF-HF) also started to diverge so that at the time of euthanasia, NF-HF weighed 16% more than NF-NF (P < 0.001), 10% more than HF-NF (P < 0.05), but still 11% less than HF-HF (P < 0.05). No differences in body weight were observed between NF-NF and HF-NF at the time of euthanasia.

On average, offspring fed a standard diet consumed 50% more food by weight than the offspring fed a high-fat/fructose diet (P < 0.001; Table 1). This lower food intake by the offspring fed a high-fat/fructose diet was also apparent when corrected for body weight (P < 0.001; Table 1). However, when corrected for energy value per gram of consumed food and fluid, even when corrected for body weight, no differences in energy intake were observed between any of the experimental groups (Table 1). Interestingly, despite no differences in energy intake between groups, HF-HF
had an average of 110% more visceral fat as a percentage of body weight at the time of euthanasia than the offspring fed a standard diet (P < 0.01; NF-NF and HF-NF) and 50% more visceral fat than NF-HF (P < 0.05; Fig. 1B).

**Effects of High-Fat/Fructose Feeding on Glucose Tolerance and Plasma Insulin**

Blood glucose levels remained similar between all experimental groups until postnatal day 91 when the offspring fed a high-fat/fructose diet (NF-HF and HF-HF) exhibited an average of 27% greater blood glucose levels than the offspring fed a standard diet (P < 0.05; NF-NF and HF-NF, Fig. 2A).

No differences in the total integrated area under the curve during oral glucose tolerance test were observed between NF-NF, NF-HF, and HF-NF groups (Fig. 2B). The area under the curve was increased on average by 15% in the HF-HF group compared with all other experimental groups (P < 0.05; Fig. 2B).

HF-NF animals had 156% (P < 0.05) and NF-HF had 229% (P < 0.01) higher plasma insulin levels compared with NF-NF (Fig. 2C). The NF-HF animals also had 39% greater insulin levels compared with HF-HF (P < 0.05), whereas no differences in insulin levels were observed between NF-NF and HF-HF groups.

**Effects of High-Fat/Fructose Feeding on Renal Function and Structure**

HF-HF had an average of 286% greater urine albumin excretion (UAE) compared with all the other treatment groups (P < 0.001; Fig. 3A). One of the mechanisms that could contribute to the increase in UAE may be increased glomerular permeability due to damage of the glomerular filtration barrier. Nestin is one of the integral components of the glomerular filtration barrier, the loss or damage of which has been shown to predispose to increased UAE (29). We find that maternal high-fat/fructose feeding, regardless of the postnatal diet, was associated with reduced nestin expression. Specifically, HF-NF and HF-HF groups were characterized by an average of 76% decrease in the expression of nestin protein, as measured by image analysis following immunohistochemistry, compared with the NF-NF and NF-HF groups (P < 0.05; Fig. 3B).

While there was no evidence of glomerular injury in the NF-NF, NF-HF, and HF-NF groups, GSI, defined as expansion of mesangial areas and dilatation of intraglomerular capillaries, was evident in the HF-HF group (Fig. 4). The index of GSI was on average 60% greater in the HF-HF group compared with treatment groups (P < 0.05; Fig. 4). No differences in TIFI were observed between any of the experimental groups (data not sown).

Kidney/body weight was on average 12% lower in the offspring fed a high-fat/fructose diet (NF-HF and HF-HF) compared with the offspring fed a standard diet (P < 0.01; NF-NF and HF-NF, Table 1). Even though no differences in MAP were observed between any of the treatment groups, there was a trend toward an increase in MAP and SBP in the

![Fig. 2. Effects of high-fat/fructose feeding on blood glucose and plasma insulin.](http://ajpregu.physiology.org/)

AJP-Regul Integr Comp Physiol • doi:10.1152/ajpregu.00433.2012 • www.ajpregu.org
offspring fed a high-fat/fructose diet (Table 1). No differences in HR or GFR were observed between any of the experimental groups (Table 1).

Effects of High-Fat/Fructose Feeding on Renal Inflammation

Transforming growth factor-β protein expression, as measured by Western blotting, was increased by an average of 100% in HF-NF, NF-HF, and HF-HF groups compared with NF-NF (P < 0.05; Fig. 5A).

CD68-positive cells, indicating the presence of macrophages, were prominent in the glomeruli and tubulointerstitial area of the renal cortex (Fig. 5B). The HF-HF group exhibited a 116% increase in the abundance of CD68-positive cells in the glomeruli and cortical tubulointerstitium compared with the NF-NF group (P < 0.05; Fig. 5B).

DISCUSSION

The aim of the present study was to examine the impact of high-fat/fructose feeding during pregnancy and postnatal development on metabolic and renal dysfunction in the female offspring later in their life. Similar to our observations in males, female offspring born to mothers fed a high-fat/fructose diet during gestation and also fed the same high-fat/fructose diet for up to 17 wk postnatally (HF-HF) were characterized by increased body weight, visceral adiposity, elevated blood glucose levels, albuminuria, glomerulosclerosis, and renal inflammation. The severity of these changes was greater than in either offspring of mothers fed a standard diet and fed a fat/fructose diet postnatally (NF-HF) or offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally (HF-NF). These observations confirm our hypothesis that high-fat/fructose feeding during pre- as well as postnatal development increases the severity of both renal and metabolic dysfunction in adulthood.

Consistent with previously published observations, we show that high-fat/fructose feeding during prenatal and continuing into postnatal development increases the risk of metabolic defects later in life (8, 14, 19). Despite no differences in birth weight between experimental groups, HF-HF offspring started to become heavier 3 wk following weaning. Thus, unlike in some models of intrauterine undernutrition such as maternal protein restriction (3, 11, 17, 31) or caloric restriction (3, 11, 31), which are often characterized by lower offspring birth weight, offspring born to high-fat/fructose fed mothers, as observed in the present study, seem to exhibit a delayed response with respect to changes in body weight. Similar observations were made in other rodent models of maternal overnutrition (4, 10, 20, 22, 27). In agreement with previous reports in both males and females, the increase in body weight in the HF-HF offspring was accompanied by increased visceral fat (8, 10, 14, 19–20) in females, despite no changes in energy intake, suggesting a reduced metabolic rate in these animals. Further studies are necessary to examine the mechanisms behind this observation as well as directly examine the effect of high-fat/fructose feeding on metabolic dysfunction in adulthood.

Fig. 3. Effects of high-fat/fructose feeding on urine albumin excretion (UAE) and integrity of the glomerular filtration barrier. A: UAE; B: nestin immunolocalization (brown staining, top). Original magnification ×400 and image analysis (bottom). Values are means ± SE. (NF-NF, n = 8; NF-HF, n = 78; HF-NF, n = 6; HF-HF, n = 6).
HF-HF on metabolic rate. In contrast to our previous study in males (14), NF-HF also exhibited increased body weight compared with the HF-NF and NF-NF offspring, however, not to the same extent as the HF-HF. This observation suggests that while high-fat/fructose feeding during postnatal development by itself affects body weight, at least in females, high-fat/fructose feeding during gestational development further adds to increases in body weight of the offspring in their adulthood in both sexes.

Along with increased body weight, the HF-HF offspring also had other metabolic abnormalities, including elevated blood glucose levels and impaired glucose tolerance. While the increase in blood glucose levels is consistent with previous observations in both males and females (5, 15, 19), the impaired glucose tolerance has not previously been reported. Despite elevated blood glucose levels and glucose intolerance, the female HF-HF offspring in this and other studies exhibited normal insulin levels (5, 15), suggesting islet cell dysfunction. Furthermore, since the elevation in glucose levels became apparent toward the end of the study, it is conceivable that extending the study for a longer follow-up period may reveal even greater increases in blood glucose levels and other metabolic disturbances leading to Type 2 diabetes. In contrast to the females, the male HF-HF offspring had normal oral glucose tolerance curves (14) with hyperinsulinemia, suggesting insulin resistance (5, 14–15, 19–20). These data demonstrate sex differences in the metabolic response to high-fat/fructose feeding, with the males being insulin resistant, while the females showing susceptibility to Type 2 diabetes. Interestingly, the female NF-HF and HF-NF offspring displayed a similar metabolic phenotype to the previously reported male offspring (14), suggesting that either maternal or postnatal high-fat/fructose feeding individually affect the metabolic health of the offspring. However, it is the combined pre- and postnatal high-fat/fructose feeding that result in a more severe metabolic phenotype, especially in females.

Similar to our previous observations in the males (14), the female HF-HF offspring developed albuminuria, podocyte injury (as measured by decreased nestin expression), and GSI, but no differences in GFR were observed. These observations suggest that albuminuria, podocyte injury, and GSI developed independent of changes in renal hemodynamics. The decrease in nestin expression leading to the loss of podocyte integrity and thus increased leakiness of the glomerular filtration barrier has been reported to be characteristic of proteinuric diseases (26). Similarly, the reduced nestin expression could explain the increased proteinuria in the HF-HF offspring. Given that activation of inflammatory pathways has been linked to both albuminuria and glomerular injury (7, 33), it is conceivable that the observed renal injury could have been triggered by renal inflammation. Indeed, we found that the HF-HF offspring exhibited increased expression of TGF-β protein along with macrophage infiltration. In contrast to numerous studies that have examined the impact of a combined pre- and postnatal high-fat/fructose feeding on metabolic function, little is known about its impact on renal function in either males or females. This is surprising given ample evidence for overweight and obesity being strong risk factors for chronic and end-stage renal disease (12, 13). In an ovine model, exposure to a high-fat diet during early postnatal development resulted in increased expression of pro-inflammatory genes in the kidney and macrophage infiltration (23). Adult offspring of Sprague-Dawley rats fed an obesogenic diet during pregnancy and suckling followed by standard chow postweaning, exhibited decreased renal renin and Na⁺-K⁺-ATPase activity (2, 21) despite no changes in kidney weight, morphology, or glomerular number (2). These studies support the notion that high-fat/fructose feeding either pre- or postnatally programs renal abnormalities later in life; however, neither of these studies examined the potential additive effect of pre- and postnatal high-fat/fructose feeding on renal health in adulthood. Our data from this and a previous study (14) attest to the fact that
high-fat/fructose feeding during both pre- and postnatal development amplifies the degree of renal dysfunction in male as well as female offspring.

Since the kidney is an important determinant of long-term blood pressure control, these studies also examined the impact of the combined effect of pre- and postnatal high-fat/fructose feeding on blood pressure in the offspring. Consistent with our previous observations in the male offspring (14), we did not observe any differences in MAP between any of the treatment groups. These observations suggest that the observed renal changes are not driven by increases in MAP. This is however in contrast to previous studies in other experimental models showing increased MAP in both males (8, 20) and females (8, 15, 20). The most likely explanation for the discrepancy in the findings of these and the present study is the duration of the follow-up: our animals were 17 wk of age at the time of euthanasia and blood pressure recording, while other studies were performed in animals of up to 36 wk of age. It should also be noted that MAP was measured in conscious animals instrumented with catheters 24 h before measurement. Thus future studies of longer duration using telemetry may determine whether increased blood pressure would develop in the offspring exposed to a high-fat/fructose diet during pre- or postnatal life.

As mentioned earlier, models of intrauterine undernutrition as a result of maternal protein restriction (3, 11, 17, 31) as well as models of maternal overnutrition (4, 10, 20, 22, 27) are associated with a cardiometabolic phenotype in the offspring. Our own study, which utilized high-fat/fructose feeding also showed a similar phenotype in the offspring. While our study further shows that the observed effects are unlikely due to differences in energy intake, it is difficult to draw conclusions whether it is the high fructose or high fat in the diet that contributed to the observed phenotype. Furthermore, the high-fat/fructose diet was also lower in protein compared with the standard diet, suggesting that lowering of the protein content may also have contributed to the cardiometabolic health of the offspring. Future studies are needed to dissect out the individual contribution of different components of the diet to the cardiometabolic phenotype in the offspring.

**Perspectives and Significance**

In summary, our data confirm our hypothesis that coupling of high-fat/fructose feeding during pregnancy with high-fat/fructose feeding during postnatal life increases the severity of renal and metabolic dysfunction of the female offspring in adulthood. These observations further support the concept of fetal programming of adult disease, with particular relevance to metabolic and renal disease associated with obesity.

**ACKNOWLEDGMENTS**

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**Fig. 5.** Effects of high-fat/fructose feeding on renal inflammation. **A**: representative immunoblot of transforming growth factor-β protein expression (top) and densitometric scans in relative optical density (ROD) expressed as a ratio of TGF-β/β-actin (bottom). **B**: CD68 immunolocalization (arrowheads, top). Original magnification ×400 and image analysis (bottom). Values are means ± SE. (NF-NF, n = 8; NF-HF, n = 78; HF-NF, n = 6; HF-HF, n = 6).
REFERENCES


