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ORIGINAL ARTICLE

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Excess sucrose intake during pregnancy programs fetal brain glucocorticoid receptor expression in female but not male C57Bl/6J mice

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Abstract

Background: Sex-specific mechanisms explaining the association between mothers with obesity and the development of obesity in children are poorly characterized. Permanent changes in fetal brain glucocorticoid receptor (GR) expression caused by exposure to overnutrition *in utero* may program aberrant energy homeostasis, thereby predisposing the offspring to obesity. This study explores sex differences in brain GR expression using an established mouse model of overnutrition during pregnancy.

Methods: Female C57Bl/6J mice were fed control (CON) or high-fat–high-sucrose (HFHS) diets. Dam cholesterol, insulin, and triglycerides were measured by colorimetric assays. Fetal corticosterone exposure was measured by placental *Abca1*, *Hsd11β1*, *Hsd11β2*, and brain *Nr3c1* (GR); *Pomc* expression measured by RT-qPCR.

Results: Female, but not male, HFHS fetuses had 46% decreased brain GR and twofold increased *Pomc* expression. There was decreased *Abca1* and *Hsd11β1* but not *Hsd11β2* expression in HFHS placentas. Caloric and sucrose intake, but not fat intake, in dams inversely correlated with fetal GR expression in both sexes. Excess sucrose consumption by dams inversely correlated with female fetal GR and directly correlated with female fetal *Pomc* expression.

Conclusions: Excess sucrose consumption in pregnant dams caused lower GR and higher *Pomc* expression in the female fetal brain. Clinical investigation of excess sucrose intake during pregnancy and its subsequent effect on hypothalamic-pituitary-adrenal axis activity and appetite in offspring may lead to novel, sex-specific obesity prevention strategies in the development of obesity in children.

KEYWORDS
brain, fetal programming, glucocorticoids, nutrition

1 | INTRODUCTION

Two-thirds of women entering reproductive age have excess weight or obesity in the United States.[1] Common obstetric complications, such as pregestational obesity, rapid gestational weight gain, and gestational diabetes, are facilitated, at least in part, by high calorie intake.[2] Overnutrition during pregnancy has been associated with placental dysfunction, offspring with obesity, and altered hypothalamic–pituitary–adrenal (HPA)-axis function throughout the lifespan.[3–5]

The hypothalamus is critical to the control of appetite and HPA-axis function.[6] Glucocorticoids, such as cortisol, and its receptor (glucocorticoid receptor [GR]) are potent regulators of fetal brain development.[4,6] Increased HPA-axis activity has been associated with the development of obesity and metabolic disease.[7,8] Human and animal studies have consistently shown that females are at greater risk for HPA-axis overactivity than males.[9–11] The Developmental Origins of Health and Disease paradigm posits that early life stressors lead to permanent changes in the structure and function of developing organs, including the brain.[12] Consumption of high fat and/or high simple carbohydrate diet has been shown to be detrimental to the structural and functional integrity of the developing brain.[13,14] Therefore, dysregulation of the HPA-axis during critical developmental timepoints may permanently alter HPA-axis feedback loops thereby predisposing offspring to abnormal basal and stress-induced function of HPA-axis throughout the lifecycle.

Increased fetal glucocorticoid exposure may downregulate GR receptor expression in the brain during critical windows of brain development thereby permanently altering the function of the HPA-axis glucocorticoid feedback loop. Previous animal studies have demonstrated that perinatal growth restriction, related to calorie or protein restriction during pregnancy, results in abnormal offspring HPA-axis activation.[4,15,16] Studies evaluating the consequences of overnutrition during pregnancy on fetal HPA-axis function, however, are sparse. Whether overnutrition during pregnancy through consumption of a high-fat–high-sucrose (HFHS) diet permanently alters fetal HPA-axis glucocorticoid feedback is unknown. Additionally, no previous studies have evaluated if a sex-specific association exists between overnutrition during pregnancy and fetal HPA-axis activation or regulation. Therefore, the primary objective of this study was to investigate sex differences in GR expression in the developing brain following exposure to HFHS overnutrition during pregnancy using an established mouse model.[5]

2 | MATERIALS AND METHODS

2.1 | Animals and diets

Animals were group housed (two pregnant dams per cage) under controlled conditions (25°C, 12-h light/dark cycle, lights on 0600 h). Female, ~6-week-old, C57Bl/6J mice (The Jackson Laboratory) were randomly allocated to be fed ad libitum either a control diet (D12489B; Research Diets, 10.6 kcal% fat, 16.8 kcal% protein, 72.6 kcal% carbohydrate, 240 g/kg sucrose) (CON group) or a HFHS diet (HFHS group). The HFHS diet consisted of both, a HFHS pellet (Western Diet D12079B; Research Diets, 40.0 kcal% fat, 17.0 kcal% protein, 43.0 kcal% carbohydrate, 340 g/kg sucrose) and 20% sucrose solution supplemented with vitamins (AIN Vitamin Mixture; MP Biomedicals) and minerals (AIN-93M Mineral Mix; MP Biomedicals) as previously described.[5] Protein content was similar between diets in order to avoid intrauterine growth restriction. Males used for breeding were fed the standard chow. All animals had free access to water.

Housing conditions, prior to mating and through gestation, were similar between CON and HFHS groups to avoid differences in isolation-associated stress. Energy consumption was measured per cage and averaged between animals in each cage. Energy consumption was measured by weighing the remaining pellets weekly and remaining sucrose solution every 48 h. An estimation of macronutrient intake (grams/day) was determined using known macronutrient content in each pellet (i.e., 72.6% carbohydrate; dam consumption 3 g/day; carbohydrate [grams/day] = 0.726 × 3 g/day).

Age-matched CON and HFHS females were mated once females in the HFHS group had gained 25% of their initial body weight (~10–12 weeks of age). Two females, in proestrus or estrus, were mated with males overnight and continued their respective diets during the mating period. Pregnancy was confirmed by the presence of a post-copulatory plug, which was defined as gestational day (GD) 0.5. Animals were maintained on their respective diets throughout gestation. A random selection of 10 CON and 10 HFHS dams from a large breeding cohort were included (N = 10/group). Litters containing fewer than five or greater than eight fetuses were excluded from fetal studies to control for variation in fetal nutrient exposure, resulting in a final cohort of 9 CON litters and 8 HFHS litters studied. Fetal sex was determined by RT-qPCR assessment of *Sry* gene amplification in three randomly chosen fetuses per litter (N = 51). One *Sry* + fetus and one *Sry*- fetus per litter were studied (CON: n = eight male, n = nine female; HFHS: n = eight male, n = seven female).

2.2 | Terminal collection of tissue and blood samples

Pregnant mice were euthanized on GD 18.5, a timepoint that corresponds to hypothalamic and hippocampal development at the beginning of third trimester in humans.[4] Dams were fasted for 4 h and euthanized individually using CO2 asphyxiation in the afternoon (1500 h) in a room separate from where other animals were housed. The body weight was measured. Blood was collected by cardiac puncture within 90 s of being euthanized. Blood was then spun at 5000 RPM for 10 min. Supernatant was collected and stored at −80° C for later analysis. Following laparotomy, fetuses and placentas were collected, remaining fetal membranes removed, tissue was dried on blotting paper and then weighed. Placentas were snap frozen for subsequent analysis. Fetuses were decapitated within 5 min following laparotomy. Fetal brain, including the pituitary gland,

was removed, weighed, and snap frozen in liquid nitrogen for subsequent analysis. All tissues were stored at −80°C for later analysis.

2.3 | Biochemical analyses

Whole blood glucose concentration (mg/dl) was measured by cardiac puncture in dams and from truncal blood in fetuses using a Contour Next glucometer (Ascensia Diabetes Care). Plasma cholesterol, insulin, triglycerides, and corticosterone were determined by colorimetric assay as previously described.[17] Insulin sensitivity was measured by surrogate measures of insulin resistance: homeostasis model of insulin resistance (HOMA-IR = fasting insulin[ng/ml] × fasting glucose[mg/dL]/22.5) and quantitative insulin check index of insulin sensitivity (QUICKI = 1/[Log(insulin) + Log(glucose)]) which have been previously validated in C57Bl/6J mice.[18]

2.4 | Gene expression analyses

Total RNA extraction and gene expression were measured from whole placenta and fetal brain using predesigned exon spanning primers utilizing the TaqMan gene expressions system (Table S1; Applied Biosystems) on a QuantStudio 3 Real-Time PCR System (Applied Biosystems) as previously described.[19] Key target genes involved in HPA-axis regulation were studied. Gene assays studied included: *Nr3c1* (gene transcript for the GR), *Pomc* (gene transcript for both proopiomelanocortin and adrenocorticotropin hormones, POMC/ACTH) in whole brain (including pituitary gland) tissue homogenates and *11β-Hsd1* or *11β-Hsd2* (genes involved in the regulation of corticosterone transfer between dam and fetus, placental stress response) and *Abca1* (gene involved in the placental cholesterol transport) in placenta tissue homogenates. *Nr3c1* and *Pomc* were measured as key targets in the fetal HPA-axis feedback loop. *11β-Hsd1*, *11β-Hsd2*, and *Abca1* were measured as key targets in placental mediated fetal corticosterone exposure.

Data were normalized to 18s rRNA using the cycle threshold (ΔΔCT) method. To confirm 18s rRNA as a suitable reference gene, expression of six common endogenous reference genes (*18s*, *Gapdh*, *Trfc*, *Actb*, *Pgk1*, *Hprt*) were evaluated in whole brain and placenta homogenates by geNorm,[20] NormFinder,[21] the comparative delta-Ct method,[22] and BestKeeper[23] using a web-based program (https://www.heartcure.com.au/reffinder/)[24] (Table S2). The ranking order of the reference genes varied between algorithms; however, 18s rRNA presented a M-value of less than 1.5 in both fetal brain and placenta tissue and therefore was considered suitable as a reference gene expression analysis between groups.[20]

2.5 | Independent and dependent variables

The primary outcome, and dependent variable, was offspring *Nr3c1* mRNA expression. The independent variable was fetal exposure to HFHS diet during pregnancy. Secondary outcomes included fetal and placental weights, placental gene expression of cholesterol transport and stress response, and fetal *Pomc* gene expression in the brain.

2.6 | Data presentation and statistical analysis

Pregnant mice on control diet and their fetuses were used as control groups. In grouped analyses, mRNA expression was reported relative to E18.5 male fetuses of pregnant mice on control diet. To determine sample size, the resource equation was used (Formula: E = total number of animals−total number of groups) where the degree of freedom (E) must be greater than 10.[25–27] Data from primary outcome groups passed the Shapiro–Wilk normality test; therefore, results were analyzed using unpaired t-test to measure group differences and two-way ANOVA followed by *post-hoc* Tukey's HSD to measure diet and sex effects between groups. *Post-hoc* was conducted only when a significant interaction between diet and sex was present in the two-way ANOVA analysis. Pearson correlation coefficient was used as a measure of linear correlation. Linear regression analysis was used to determine goodness of fit for significant linear correlations. Statistical analysis and graphics were performed using GraphPad Prism version 7 (GraphPad Software, Inc). Data are presented as mean ± SEM. N represents the number of animals per group. A p value less than 0.05 was considered statistically significant.

IACUC Approval: All protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee.

3 | RESULTS

3.1 | Characteristics of pregnant dam and fetuses

Group differences in weights, food consumption, and metabolic profile in pregnant dams; fetal weights and litter size are provided in Table 1. HFHS dams were 8% heavier at mating compared with CON dams. Body weight of dams at GD 18.5 did not differ between groups. HFHS female fetuses were 11% lighter compared with CON female fetuses. There was no difference in weight between CON and HFHS male fetuses. Litter sizes between CON and HFHS groups did not differ.

Pregnant HFHS mice had 33% greater energy intake compared with CON mice ($p < 0.0001$). The increased energy consumption in HFHS group was due to 3.4-fold higher sucrose intake ($p < 0.0001$) and 3.0-fold higher fat intake ($p < 0.0001$). The HFHS group had 22% higher total carbohydrate intake compared with CON mice; however, the majority of carbohydrate intake in the HFHS dam was sucrose (35% CON vs. 90% HFHS, $p < 0.0001$). In the HFHS group, 59% of sucrose intake was due to sucrose solution with the remaining 41% by pellet (data not shown). There was no difference in protein intake between CON and HFHS pregnant mice.

Fasting blood glucose, insulin, and triglyceride levels were measured at GD 18.5 as a marker of glucose tolerance and diabetes

TABLE 1 Characteristics of cohort

	CON	HFHC	p value	Diet and sex effect		
Maternal						
Body weight at mating, g	21.32 + 0.43	23.30 + 0.58	0.01			
Body weight at GD 18.5, g	34.20 + 1.1	36.32 + 1.3	NS			
Energy intake, kcal/kg/day	548.2 + 15.88	818.1 + 28.57	0.0001			
Total carbohydrate intake, g/day	2.06 + 0.13	2.63 + 0.11	0.003			
Sucrose intake, g/day	0.71 + 0.03	2.37 + 0.10	<0.0001			
Fat intake, g/day	0.39 + 0.07	1.14 + 0.05	<0.0001			
Protein intake, g/day	0.50 + 0.02	0.48 + 0.02	NS			
Glucose, mg/dl	121.5 + 7.51	126.1 + 6.16	NS			
Insulin, ng/ml	0.98 + 0.28	1.08 + 0.34	NS			
HOMA-IR	3.69 + 0.39	4.27 + 1.03	NS			
QUICKI	0.52 + 0.02	0.52 + 0.03	NS			
Triglycerides, mg/dl	63.45 + 4.32	53.08 + 3.11	0.06			
Cholesterol, mg/dl	28.68 + 2.55	62.83 + 5.52	<0.0001			
Corticosterone, ng/ml	223.6 + 47.99	116.6 + 13.68	0.04			
Fetal				Interaction	Diet	Sex
Weight (male), g	1.131 + 0.02	1.101 + 0.04	NS	NS	0.02	NS
Weight (female), g	1.159 + 0.02	1.029 + 0.05	0.02			
Glucose (male), mg/dl	64.44 + 5.70	48.38 + 9.09	NS	NS	NS	NS
Glucose (female), mg/dl	49.88 + 6.14	47.14 + 5.72	NS			
Litter size, #	7.3 + 0.40	6.5 + 0.54	NS			

Note: Results are expressed as mean + SEM, p values > 0.1 recorded as NS. Maternal characteristics recorded on GD 18.5, n = 8–9/group. Individual fetal characteristics recorded on E18.5, n = 7–9/group. Fetal weights are an average of fetuses included from separate litters rather than a mean weight of each litter. Group differences measured by upaired t-test, diet and sex effects measured by two way ANOVA, mice born to dams consuming same diet (diet effect), versus male mice born to dams consuming different diets (sex effect).

Abbreviations: GD, gestational day; HOMA-IR, homeostasis model index of insulin resistance; NS, nonsignificant; QUICKI, quantitative insulin-sensitivity check index.

in the pregnant dam. HOMA-IR and QUICKI were calculated as surrogate measures of insulin resistance and diabetes. There was no difference in these measurements between the CON and HFHS groups. HFHS dams had 2.2-fold higher serum cholesterol concentration compared with CON dams ($p < 0.0001$). Fetal blood glucose was measured at E18.5 as a proxy for fetal glucose homeostasis and stress. There were no differences between the CON and HFHS groups in either male or female fetuses. Stress response in dams was measured at GD 18.5 by plasma corticosterone. Dams exposed to HFHS diet had 52% lower plasma corticosterone compared with control dams.

3.2 | Fetal HPA-axis regulation

Group differences, effects of diet and sex, and relationships between key targets of the HPA-axis on E18.5 are shown in Figure 1. Relative mRNA expression of GR and *Pomc/Acth* were significantly reduced in the brain of female fetuses in the HFHS group, but not in males. GR mRNA expression was 46% lower ($p = 0.03$) and *Pomc/Acth* mRNA expression was 2.0-fold higher ($p = 0.02$) in female fetuses in the HFHS group, compared with CON females. Brain GR mRNA expression showed a strong inverse correlation with *Pomc/Acth* mRNA expression ($r = -0.52$, $p = 0.004$).

To investigate sex differences in HPA-axis activity (decreased GR and increased *Pomc/Acth*), fetal-placental weights and key targets involved in placental-mediated corticosterone exposure were measured. As discussed above, female HFHS fetuses were 11% lighter than CON females (Table 1). There was no significant difference in female placental weight between the CON and HFHS groups (Figure 2). Additionally, there were no differences between placental *11β-Hsd1*, *11β-Hsd2*, or *Abca1* mRNA expression between CON and HFHS males or females (Figure 3). Potential relationships between fetal HPA-axis targets and characteristics of dam, placenta, and fetus are described in Table 2. Energy consumption, specifically carbohydrate consumption, in dams during pregnancy

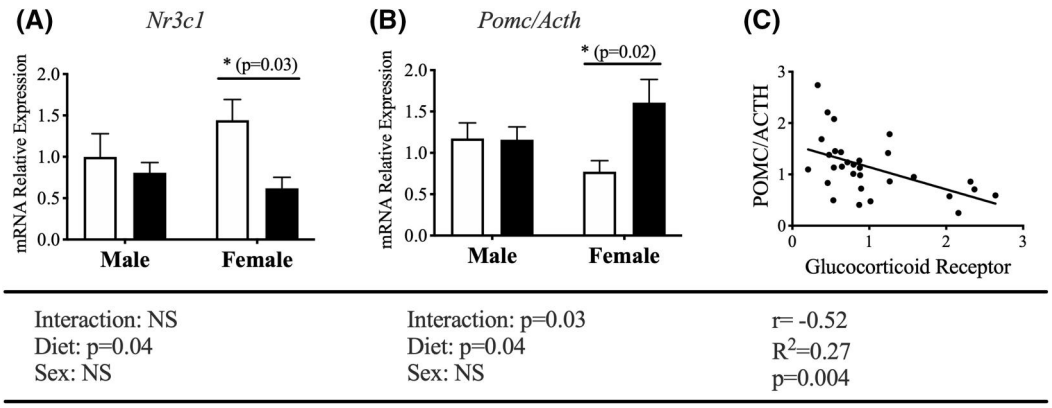

FIGURE 1 Overnutrition during pregnancy was associated with lower glucocorticoid receptor and higher *Pomc/Acth* transcript expression in the female fetal brain. *Nr3c1* (gene transcript for the glucocorticoid receptor) and *Pomc* (gene transcript for both proopiomelanocortin and adrenocorticotropin hormones) were measured in embryonic day (E) 18.5 male and females exposed to dams on control (CON) and high-fat-high-sucrose (HFHS) diets during gestation. E18.5 HFHS females had 46% lower *Nr3c1* (Panel A, $p = 0.03$) and 2-folds higher *Pomc* (Panel B, $p = 0.03$) mRNA expression in the brain compared with CON females. *Nr3c1* and *Pomc mRNA* expression showed a strong inverse relationship (Panel C, $p = 0.004$). Diet, not sex, effected *Nr3c1* mRNA expression (Panel A, diet effect $p = 0.04$). Diet had more of an effect on *Pomc mRNA* expression in females compared with males (Panel B, diet effect $p = 0.04$, interaction between diet/sex $p = 0.03$). Panel A & B: Results are expressed as mean ± SEM; open bars are E18.5 offspring born to dams on control diet; closed bars are E18.5 offspring born to dams on HFHS diet; Two-way ANOVA followed by *post-hoc* Tukey's HSD, $n = 6$–8/group, values obtained at E18.5. versus mice born to dams consuming same diet (diet effect), versus male mice born to dams consuming different diets (sex effect), differences between CON and HFHS exposed female offspring were significant (*$p = 0.03$ in Panel A and *$p = 0.02$ Panel B). Panel C: results expressed as correlation coefficient (r) and linear regression (R^2). *p*-Values >0.1 recorded as nonsignificant (NS)

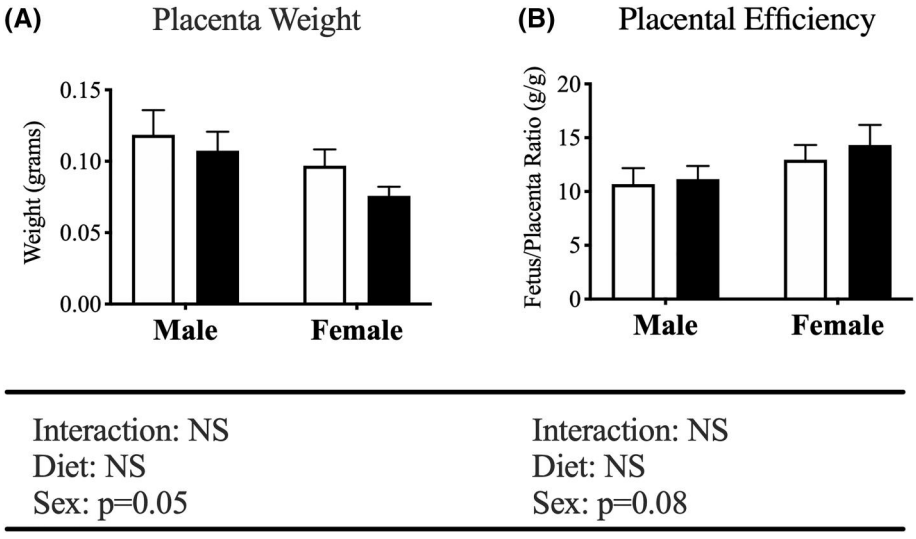

FIGURE 2 Overnutrition during pregnancy was associated with a trend towards lower placenta weight and higher placental efficiency. Placenta and fetal weights were measured in embryonic day (E) 18.5 placentas from dams on control (CON) and high-fat-high-sucrose (HFHS) diets during gestation. Fetal weight/placenta weight ratio (F/P) were used as a surrogate for placental efficiency. E18.5 female placentas had a trend towards lower placental weight (Panel A, $p = 0.05$) and higher F/P ratio (Panel B, $p = 0.00$) compared with male placenta. There were no differences in placenta weight or F/P ratio between CON and HFHS placenta. There was no interaction measured between diet and sex in groups measured. Results are expressed as mean ± SEM; open bars are CON E18.5 offspring; closed bars are HFHS E18.5 offspring; Two-way ANOVA followed by *post-hoc* Tukey's HSD, $n = 6$–8/group, values obtained at E18.5. versus mice born to dams consuming same diet (diet effect), versus male mice born to dams consuming different diets (sex effect). *p*-Values >0.1 recorded as nonsignificant (NS)

showed a strong inverse relationship with fetal brain GR mRNA expression in both sexes (Figure 2, $r = -0.59$, $p = 0.005$ for energy consumption; $r = -0.61$, $p = 0.0004$ for carbohydrate consumption). Female fetal brain GR mRNA expression demonstrated a strong direct correlation with placental weight ($r = 0.63$, $p = 0.02$) and a strong inverse correlation with fetal weight/placental weight ratio, which is a proxy for placental efficiency ($r = -0.57$, $p = 0.03$) (Table 2).

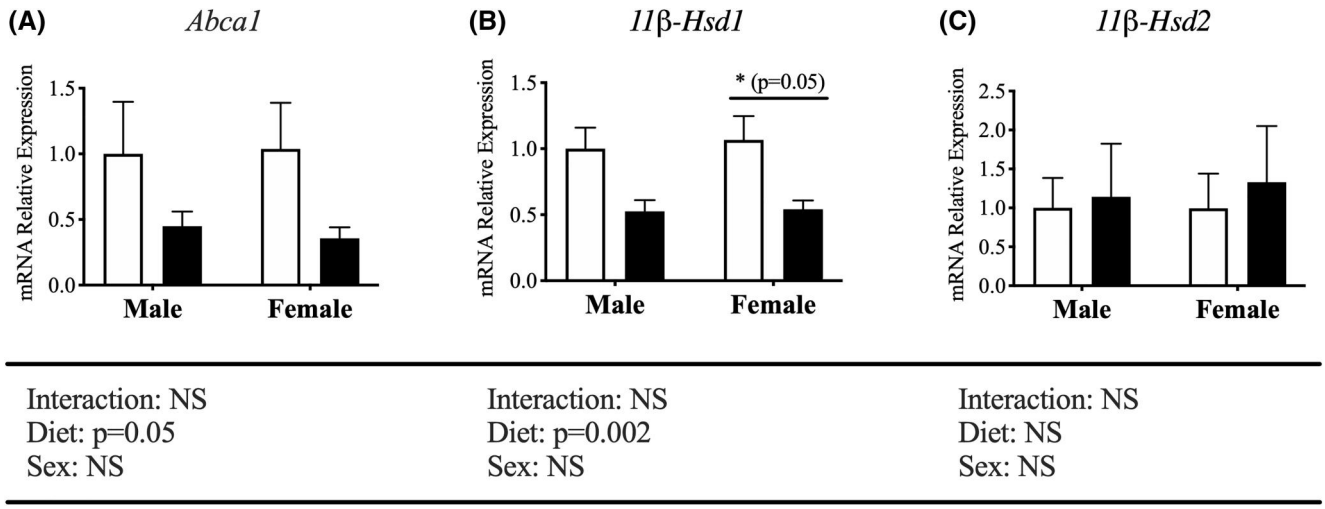

FIGURE 3 Overnutrition during pregnancy was associated with lower *Abca1* and *11β-hsd1* but not *11β-hsd2* transcript expression in the placenta. *Abca1* (gene transcript for placental cholesterol transport) and *11β-hsd1* (gene transcript for enzyme which activates corticosterone), and *11β-hsd2* (gene transcript for enzyme which inactivates corticosterone) were measured in embryonic day (E) 18.5 placentas from dams on control (CON) and high-fat-high-sucrose (HFHS) diets during gestation. E18.5 HFHS placentas had 60% lower *Abca1* (Panel A, $p = 0.05$) and 46% lower *11β-hsd1* (Panel B, $p = 0.002$) mRNA expression compared with CON. *11β-hsd1* mRNA expression was 49% lower in HFHS females compared with female CON fetuses ($p = 0.05$). There were no significant differences noted between CON and HFHS males on post-hoc analysis. There were no differences between CON and HFHS placenta *Abca1* mRNA expression in male or female subgroups in post-hoc analysis. There was no significant difference between HFHS and CON placenta in *11β-hsd2* mRNA expression (Panel C). There was no sex specific effect in mRNA expression of Abca1, *11β-hsd1*, and *11β-hsd2* between males and female placenta. There was no interaction measured between diet and sex in groups measured. Results are expressed as mean ± SEM; open bars are E18.5 placenta exposed to control diet; closed bars are placenta exposed to HFHS diet; Two-way ANOVA followed by *post-hoc* Tukey's HSD, $n = 6$–8/group, values obtained at E18.5 versus mice born to dams consuming same diet (diet effect), versus male mice born to dams consuming different diets (sex effect). *p*-Values >0.1 recorded as nonsignificant (NS)

Weight, cholesterol levels, or the metabolic profile of the pregnant dam did not correlate with fetal brain GR mRNA expression (Table 2). Similarly, there was no relationship between fat consumption in pregnant dams and fetal GR mRNA expression (Figure 4), despite a significant increase in fat consumption by the HFHS pregnant dams (Table 1). Placental cholesterol transport (*Abca1*) in female placentas strongly and directly correlated with female GR ($r = 0.65$, $p = 0.02$) and inversely with *Pomc/Acth* ($r = -0.56$, $p = 0.047$) mRNA expression in the brain (Table 2). Placental stress response (*11β-Hsd1*, *11β-Hsd2*) or fetal characteristics did not correlate with GR mRNA expression in the brain (Table 2). There were no relationships between any of the other measured characteristics described in the pregnant dam, placenta, or fetus and *Pomc/Acth* mRNA expression in the fetal brain (Table 2).

3.3 | Diet during pregnancy versus fetal sex and brain glucocorticoid mRNA expression

To explore a potential mechanism explaining lower GR mRNA expression in female fetal brains, the effects of diet and fetal sex on the components of the fetoplacental unit were analyzed. In relation to the fetus, diet during pregnancy had a significant effect on fetal body weight (Table 1, $p = 0.02$). Placental *11β-Hsd1* mRNA expression was also affected by diet during pregnancy (Figure 3, $p = 0.002$). Placenta *11β-Hsd1* mRNA expression was 49% lower in HFHS females compared with the CON fetuses ($p = 0.05$); however, there were no significant differences noted between CON and HFHS males on post-hoc analysis. There was a trend of lower placental *Abca1* mRNA expression (60% lower) in HFHS fetuses without differences between male or separate female groups on post-hoc analysis (Figure 3, $p = 0.05$). Fetal blood glucose levels (Table 1), placental weight (Figure 2), fetal weight/placental weight ratio (Figure 2), and *11β-Hsd2* mRNA expression (Figure 3) were not affected by diet during pregnancy.

The only population characteristic affected by fetal sex was in the placenta. Placental weight trended lower in CON and HFHS females compared with CON and HFHS males (Figure 2, $p = 0.05$). Fetal weight/placental weight ratio also trended higher in CON and HFHS females compared with CON and HFHS males (Figure 2, $p = 0.08$). There were no significant interactions between fetal sex and brain GR or *Pomc/Acth* mRNA expression (Figure 1). Additionally, there was no significant interaction between fetal sex, fetal weight or glucose and brain GR or *Pomc/Acth* mRNA expression (Figure 1). There were no significant interactions between fetal sex and targets of placental stress response (*11β-Hsd1*, *11β-Hsd2*) or cholesterol (*Abca1*) transfer (Figure 2).

TABLE 2 Maternal-placental-fetal associations with HPA-axis in the female brain

	Glucocorticoid receptor		POMC/ACTH hormone	
	Correlation coefficient (*r*)	*p* value	Correlation coefficient (*r*)	*p* value
Maternal				
Body weight at mating, g	−0.40	NS	0.23	NS
Body weight at GD 18.5, g	−0.16	NS	−0.05	NS
Energy intake, kcal/kg/day	−0.59	0.02	0.64	0.01
Carbohydrate intake, g/day	−0.68	0.005	0.40	NS
Sucrose intake, g/day	−0.56	0.03	0.56	0.03
Fat intake, g/day	−0.33	NS	0.52	0.048
Protein intake, g/day	−0.33	NS	0.04	NS
Glucose, mg/dl	0.01	NS	0.09	NS
Insulin, ng/ml	0.23	NS	0.23	NS
Triglycerides, mg/dl	0.25	NS	0.25	NS
Cholesterol, mg/dl	−0.40	NS	−0.15	NS
Placental				
Weight, g	0.63	0.02	−0.29	NS
Fetal/placental ratio (g/g)	−0.57	0.03	0.24	NS
11b-Hsd1 expression	0.32	NS	0.23	NS
11b-Hsd2 expression	0.10	NS	0.15	NS
Abca1 expression	0.65	0.02	−0.56	0.047
Fetal				
Weight, g	0.30	NS	0.20	NS
Brain/body weight ratio (g/g)	−0.02	NS	−0.16	NS
Blood glucose (mg/dl)	0.25	NS	0.28	NS
Litter size (#)	0.002	NS	−0.15	NS

Note: Results are expressed as correlation coefficient (*r*), *p* values > 0.1 recorded as NS. Characteristics of pregnant dam and placenta recorded on gestational day 18.5, *n* = 8–9/maternal group, *n* = 6–8/placental group. Characteristics of female fetuses recorded on embryonic day 18.5, *n* = 7–9/group.

Abbreviations: GD, gestational day; HPA, hypothalamic-pituitary-adrenal; NS, nonsignificant.

4 | DISCUSSION

This study adds to the currently sparse reports exploring sex differences in fetal HPA-axis regulation following exposure to overnutrition during pregnancy. Exposure to HFHS diet increased caloric intake in pregnant dams and caused lower fetal GR expression in female C57Bl/6J mouse brain. HFHS dams were not diabetic but had evidence of metabolic disease characterized by an increase in body weight at mating and higher serum cholesterol. Excess carbohydrate, primarily sucrose, consumption was the only dietary parameter that correlated with fetal GR expression. This finding suggested that excess sucrose consumption during pregnancy, rather than fat consumption or metabolic disease, was likely responsible for early programming changes in HPA-axis development.

Female fetuses exposed to HFHS diet during pregnancy had 46% decrease in GR expression and 2.0-fold increase in *Pomc/Acth* mRNA expression suggestive of altered HPA-axis regulation. This key finding was consistent with previous work regarding HPA-axis development. Transgenic mouse models have shown that a similar magnitude (30%–50% lower expression) of reduction in GR expression is associated with an exaggerated HPA response to stress.[28,29] Increased sucrose intake in the postnatal period, through chronic sucrose consumption in 3–15-week-old female rats, also resulted in lower GR gene expression in the brain (40% lower GR expression in the hippocampus).[30] Increased caloric intake, through excess standard chow, during pregnancy altered sensitivity to ACTH stimulation in 19-month ovine offspring.[31] Increased caloric intake, through HFHS during pregnancy, increased hypothalamic corticotropin-releasing hormone (*Crh*) expression and decreased hypothalamic GR expression in 5-week peripubertal female mice.[17] Although the present study was consistent with the current literature, it was also unique in that it reported sex specific hypothalamic GR mRNA

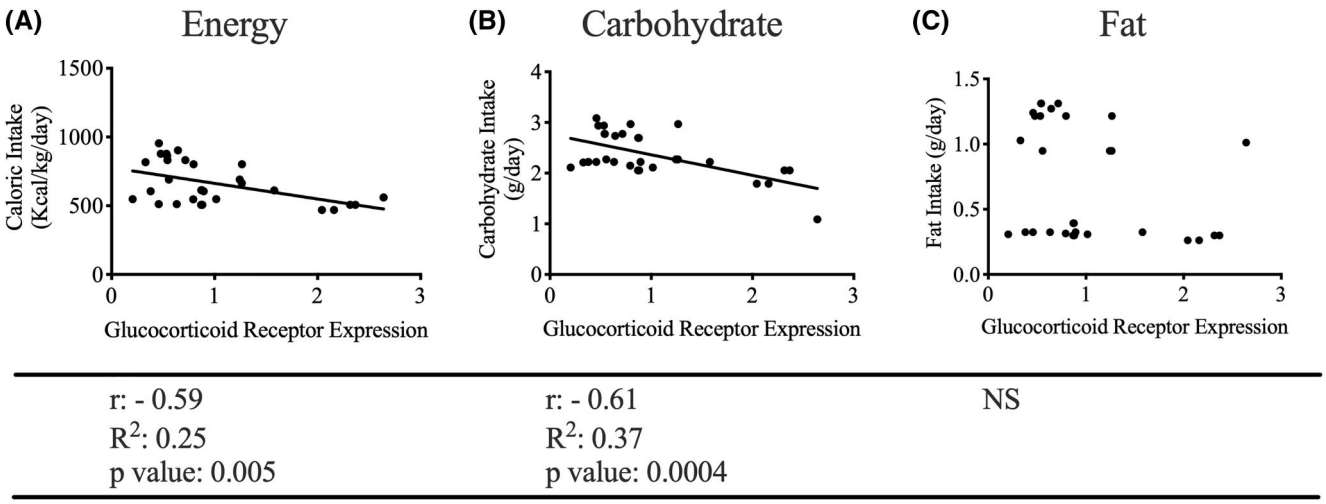

FIGURE 4 Sucrose consumption during pregnancy correlated with glucocorticoid receptor transcript expression in the fetal brain. Consumption of caloric intake was measured throughout pregnancy in dams on control (CON) and high-fat-high-sucrose (HFHS) diets. *Nr3c1* (gene transcript for glucocorticoid receptor) mRNA expression was measured in embryonic day 18.5 brains. Dam energy consumption (Panel A, $r = -0.59$) and sucrose consumption (Panel B, $r = -0.61$) demonstrated a strong inverse correlation with fetal *Nr3c1* mRNA expression in the brain ($p < 0.01$). Dam fat consumption (Panel C) was not correlated with fetal *Nr3c1* mRNA expression in the brain. The correlation between dam protein consumption and fetal brain *Nr3c1* mRNA expression was not measured as dam protein consumption was equal between CON and HFHS dams during gestation. Results are expressed as correlation coefficient (r) and linear regression (R^2), $N = 30$, *p*-values >0.1 recorded as nonsignificant (NS)

expression during brain development, rather than later timepoints, following exposure to an obesogenic diet during pregnancy.

The results of this study, highlighting changes in hypothalamic GR and *Pomc* expression only in females, represents a frameshift in the development of obesity hypothesis that has typically shown male animals to be more susceptible to obesity following exposure to high-fat diets.[32–34] Clinically, women are more susceptible to development of obesity than men.[35,36] Therefore, it remains unclear whether the inconsistency between animal models and human epidemiology is a bias of reporting male-only or mixed sex cohort outcomes or if there are sex-specific critical mechanisms that have not yet been elucidated for the development of obesity due to environmental programming changes. Although male and female offspring may share a similar phenotype of obesity in adulthood following exposure to maternal overnutrition, the programming mechanisms leading to the development of this phenotype may differ between the sexes. Further research in this area is needed to target sex specific prevention strategies in the development of obesity.

4.1 | Sex differences in HPA-axis development

Studies evaluating overnutrition during pregnancy and sex differences in fetal HPA-axis activity are limited. There were, however, several human and animal studies demonstrating that females were more vulnerable to HPA-axis programming and reactivity compared with males.[9–11,37] Carpenter and colleagues performed a systematic review of studies in humans supporting that aberrant offspring HPA-axis activity was related to prenatal stressors such as low birth weight, preterm birth, psychosocial stress during pregnancy, and glucocorticoid exposure from mother to her fetus.[10] Additionally, they reported that female placentas had increased permeability to glucocorticoids, mediated by 11β-HSD enzymes, exposing female offspring to higher exogenous GRs prenatally compared with males.[10] This review did not evaluate diet during pregnancy as a contributor to early HPA-axis activation.

Mechanisms contributing to sex differences in HPA-axis activation following stress remain under investigation. In rodent studies, females showed a more robust neuroendocrine response to stressors when compared with males.[11] Specifically, female rats have been shown to have greater expression of *Pomc* in the pituitary,[38,39] delayed return to baseline of ACTH following acute stress,[38] lower neuronal GR binding,[40] and depressed glucocorticoid feedback mechanisms[38] that result in HPA-axis overactivity compared with males. These studies support the findings reported in the present study that females had increased HPA-axis activity compared with males following prenatal stress induced by overnutrition during pregnancy.

4.2 | Sucrose and HPA-axis development

Although previous studies have investigated the impact of stress during pregnancy and offspring neuronal GR expression, few have considered the impact of diet, specifically high sugar rather than fat consumption, during pregnancy and its impact on the development of obesity in offspring.[41] Animal studies have shown that sucrose causes feedback inhibition in the hippocampus and prefrontal cortex which

downregulates hypothalamic and pituitary GR expression.[41] This mechanism may also be the case following fetal exposure to a high sucrose diet during pregnancy; however, this has not been specifically studied. This finding supported this study's hypothesis. A study aimed at comparing high-fat versus high-sugar diet in pregnancy and its impact on regional GR expression in the brain is necessary for confirmation.

An alternative mechanism to explain the role of excess sucrose consumption during pregnancy and lower fetal GR expression in the brain is the role of sucrose in fetal cholesterol production. As cholesterol is the primary substrate for cortisol production, it is possible that increased fetal exposure to cholesterol may cause increased fetal cortisol production and therefore decreased GR expression. Fetal GR mRNA expression was strongly correlated with placental cholesterol transport (*Abca1*, $r = 0.65$, $p = 0.02$) but not correlated to cholesterol in pregnant dams or placental stress response (Table 2). Placental *Abca1* expression in females was strongly and inversely correlated with carbohydrate, but not fat, consumption ($r = -0.81$, $p < 0.0001$). Further study of the effect of macronutrients on placental cholesterol transport, as well as fetal cholesterol metabolism, would be necessary to validate this speculation.

5 | CONCLUSIONS

A strength of this study is its clinical relevance to understanding the impact of diet during pregnancy on fetal brain development without being confounded by diabetes. Diabetes, in the absence of obesity, has been shown to alter hypothalamic development in rodent models.[4] Dams exposed to a HFHS diet were heavier at mating and had elevated cholesterol levels but normal fasting glucose, triglycerides, and insulin levels. Collectively, these findings suggest that dams receiving HFHS diet were insulin sensitive. This metabolic profile is clinically relevant to the large population of obstetric women who have excess weight or obesity with a normal fasting glucose and insulin sensitivity.[42] A high fat, high simple carbohydrate diet is prevalent in developed countries, thereby likely to play a central role in the obesity epidemic in these countries.[13] The majority of clinical and preclinical research to date has focused on fetal programming related to pregnant women with diabetes or obesity rather than diet during pregnancy.[3]

There are some limitations to interpreting the results of this study. First, outcomes extrapolated from animal models to the human condition must be interpreted with caution. For example, there are differences in the developmental time course of brain regions among species. This study acknowledges and leverages some of these differences; however, it is impossible to control for all biological differences between species. Results are meant to inform transitional studies to determine ultimate clinical relevance. Second, the contribution of male exposure to CON or HFHS diets during mating was not studied. A recent study reported paternal diet mediated changes in sperm and/or seminal plasma associated with obesity and poor cardiometabolic health in offspring.[43] This study reported results after males had been exposed to diets for longer durations, such as 8 weeks, rather than overnight as in this study.[43] However, the contribution of short-term diet exposure in stud males cannot be determined without additional investigation. Third, the strong correlation between fetal GR and *Pomc/Acth* expression found in this study (Figure 1) supports HPA-axis activation; however, without region-specific evaluation at the protein level, conclusions remain speculative. Additionally, POMC is a neuropeptide included in hypothalamic mediated satiety signaling as well as a precursor for ACTH, melanocyte stimulating hormone, and β-endorphin. Tissue homogenates in this study included both brain and pituitary gland; therefore, the relationship between *Pomc* and GR reflects HPA-axis regulation is conjectural. A causal relationship cannot be determined without evaluation of hormone secretion. Finally, the functional relevance of the abnormal HPA-axis activation on future risk of obesity and metabolic disease cannot be determined without additional studies.

In summary, overnutrition during pregnancy, specifically excess sucrose consumption, decreased brain GR expression in C57Bl/6J females. Altered female placental function, potentially related to cholesterol transport, may explain sex-specific differences in brain GR expression. Further gain/loss of function studies investigating regional brain GR signaling and HPA-axis glucocorticoid feedback loop activation, in HFHS diet during pregnancy exposed offspring, may further characterize a critical mechanistic link between overnutrition during pregnancy and development of obesity in offspring. Demonstration of similar effects in humans will confirm the potential link between diet during pregnancy and early fetal programming of HPA-axis regulation. Further studies are likely to offer a path for diet modification as a preventive strategy against adult HPA-axis overactivity and development of obesity.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Megan E. Paulsen: Conceptualization, methodology, formal analysis, writing—original draft preparation, funding acquisition, investigation, resources, project administration, supervision, writing—review, and editing. Debra Kulhanek: Investigation. Raghavendra B. Rao: Resources, project administration, supervision, writing—review, and editing. All authors have read and agreed to the published version of the manuscript.

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