

## OBSTETRICS

# Males are from Mars, and females are from Venus: sex-specific fetal brain gene expression signatures in a mouse model of maternal diet-induced obesity

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**BACKGROUND:** Maternal obesity is associated with adverse neurodevelopmental outcomes in children, including autism spectrum disorders, developmental delay, and attention-deficit hyperactivity disorder. The underlying mechanisms remain unclear. We previously identified second-trimester amniotic fluid and term cord blood gene expression patterns suggesting dysregulated brain development in fetuses of obese compared with lean women.

**OBJECTIVE:** We sought to investigate the biological significance of these findings in a mouse model of maternal diet-induced obesity. We evaluated sex-specific differences in fetal growth, brain gene expression signatures, and associated pathways.

**STUDY DESIGN:** Female C57BL/6J mice were fed a 60% high-fat diet or 10% fat control diet for 12–14 weeks prior to mating. During pregnancy, obese dams continued on the high-fat diet or transitioned to the control diet. Lean dams stayed on the control diet. On embryonic day 17.5, embryos were weighed and fetal brains were snap frozen. RNA was extracted from male and female forebrains (10 per diet group per sex) and hybridized to whole-genome expression arrays. Significantly differentially expressed genes were identified using a Welch's *t* test with the Benjamini-Hochberg correction. Functional analyses were performed using ingenuity pathways analysis and gene set enrichment analysis.

**RESULTS:** Embryos of dams on the high-fat diet were significantly smaller than controls, with males more severely affected than females ( $P = .01$ ). Maternal obesity and maternal obesity with dietary change in pregnancy resulted in significantly more dysregulated genes in male vs female fetal brains (386 vs 66,  $P < .001$ ). Maternal obesity with and without dietary change in pregnancy was associated with unique brain gene expression signatures for each sex, with an overlap of only 1 gene. Changing obese dams to a control diet in pregnancy resulted in more differentially expressed genes in the fetal brain than maternal obesity alone. Functional analyses identified common dysregulated pathways in both sexes, but maternal obesity and maternal dietary change affected different aspects of brain development in males compared with females.

**CONCLUSION:** Maternal obesity is associated with sex-specific differences in fetal size and fetal brain gene expression signatures. Male fetal growth and brain gene expression may be more sensitive to environmental influences during pregnancy. Maternal diet during pregnancy has a significant impact on the embryonic brain transcriptome. It is important to consider both fetal sex and maternal diet when evaluating the effects of maternal obesity on fetal neurodevelopment.

**Key words:** diet, fetal programming, growth, maternal obesity, neurodevelopment, sex differences, transcriptome

Maternal obesity has reached epidemic proportions in the United States. More than one third of reproductive-age women are obese at conception, with a 70% increase in pre-pregnancy obesity in recent decades.<sup>1,2</sup> Data from large epidemiological studies suggest an association between maternal obesity and adverse neurodevelopmental outcomes in children, including lower

general cognitive capabilities,<sup>3</sup> increased prevalence of autism spectrum disorders,<sup>4</sup> and increased prevalence of attention-deficit hyperactivity disorder.<sup>5</sup> Yet the mechanisms by which maternal obesity results in adverse neurodevelopmental outcomes for offspring remain unclear.

In our prior functional genomic analyses of second-trimester amniotic fluid supernatant and term umbilical cord blood in fetuses of obese vs lean women, we found gene expression patterns consistent with dysregulated brain development and dysregulated inflammatory and metabolic signaling.<sup>6,7</sup> We transitioned to a mouse model of maternal diet-induced obesity (DIO) to evaluate the biological significance of these findings via direct examination of the fetal brain.

In our analyses of human amniotic fluid supernatant and term cord blood, subjects were matched for fetal sex, and sex-specific effects of maternal obesity on the fetus were not evaluated. Given that some animal model studies suggest sex-specific effects of maternal obesity on offspring neurodevelopment<sup>8–11</sup> and different susceptibilities of male and female offspring to maternal dietary interventions,<sup>8</sup> here we examined both male and female embryonic brains.

Our objective was to determine whether maternal obesity is associated with a distinct pattern of fetal brain gene expression and whether maternal obesity has different effects on male and female fetal brain development by characterizing embryonic brain gene expression signatures and associated

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pathways in a mouse model of diet-induced obesity.

## Materials and Methods

### Animal model and genotyping

The Tufts Medical Center Institutional Animal Care and Use Committee approved this protocol (number B2013-82); all institutional guidelines for animal care and use were followed.

Five week old female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were fed ad libitum either a lard-based, high-fat diet (HFD) containing 60% calories from fat ( $n = 22$ ; D12492; Research Diets, New Brunswick, NJ) or a control diet (CD) containing 10% calories from fat ( $n = 10$ ; D12450; Research Diets). The diets were matched for protein, fiber, sucrose, and micro-nutrient content (Supplemental File 1).

Female mice were weighed weekly during the 12–14 week feeding period. Maternal obesity was defined as at least a 30% increase in weight compared with age-matched controls.<sup>12,13</sup> The male C57BL/6J mice were fed the CD. Prior to breeding, male and female body composition was characterized by EchoMRI (EchoMRI LLC, Houston, TX).

Obese and lean females were bred with control males. The presence of a vaginal plug was defined as embryonic day (e) 0.5. Females were weighed at pregnancy day (P) 0, P10 and P15. To separate the fetal effects of prepregnancy obesity from the effect of exposure to a high-fat diet during pregnancy, the following 3 study groups were formed:

(1) C57BL/6J female mice fed a CD throughout (CD group); (2) C57BL/6J female mice fed a HFD to induce a DIO phenotype and then continued on a HFD during gestation (HFD/HFD); and (3) C57BL/6J female mice fed a HFD to induce a DIO phenotype and then switched at e0.5 to a CD for gestation (HFD/CD).

At e17.5, pregnant mice were euthanized with isoflurane followed by decapitation. Embryos were rapidly dissected from the uterine horns and placed in ice-cold phosphate-buffered saline (1 time) containing an ribonucleic acid (RNA) preservative (RNALater;

QIAGEN, Valencia, CA). Crown-rump lengths and weights were recorded. Sex genotyping was performed on tail snip DNA using real-time PCR with specific probes for the *Sry* gene (Transnetyx, Cordova, TN).

Colony statistics were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). Male and female embryos were analyzed separately, with differences between the 3 diet groups determined using Kruskal-Wallis testing, followed by pairwise comparisons if significance was identified (Bonferroni-corrected  $P < .05$ ).

### Forebrain RNA isolation and microarray methods

Forebrains were rapidly dissected from skulls and snap frozen in liquid nitrogen. Total RNA was isolated using the NucleoSpin RNA/protein kit (Macherey-Nagel, Düren, Germany). RNA purity, integrity, and quantity were assessed using the NanoDrop ND-8000 (NanoDrop, Wilmington, DE) and the bioanalyzer system (Agilent 2100; Agilent Technologies Inc, Palo Alto, CA).

RNA samples were processed using the Affymetrix GeneChip WT PLUS kit and hybridized to Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA). Ten arrays per sex per experimental group were used, with each array corresponding to 1 animal. Four to 6 litters were represented in each diet group to minimize litter effects. Quality control and normalization were performed using the pipeline ([www.arrayanalysis.org](http://www.arrayanalysis.org); Maastricht University, The Netherlands).<sup>14</sup>

Normalization was performed using the robust multichip average algorithm<sup>15</sup> and the default Affymetrix chip description file for this chip. The 27,619 main probe sets were used for further analysis; probe sets corresponding to Affymetrix controls or unmapped sequences were discarded after normalization.

### Bioinformatics analysis of microarray data

Statistical analyses were performed using R software version 3.1.2. Male and female gene expression data were analyzed

separately. Welch's *t* tests were used to identify differentially expressed genes (DEGs) between diet groups.

*P* values were corrected for multiple testing by calculating the Benjamini and Hochberg false discovery rate (FDR).<sup>16</sup> Probe sets with an FDR  $< 20\%$  were considered significantly differentially expressed. The number of DEGs per comparison was visualized as a Venn diagram.

Gene expression changes were further visualized as a heat map combined with hierarchical clustering analysis using Euclidean distance and Ward linkage. Principal component analyses were performed using R software.

Further in silico functional analysis was performed on the top 1% of up- or downregulated genes using ingenuity pathway analysis (IPA; Ingenuity Systems, Core Analysis build version 338830M, content version 23814503). Statistical significance within the IPA was determined according to recommended thresholds ( $P < .05$  or bias-corrected absolute *Z* score  $\geq 2$ ).<sup>17–19</sup> Only pathways containing 3 or more genes were considered. The microRNA Target Filter tool on the IPA was used to predict genes and pathways affected by dysregulated microRNAs. Only high-confidence predictions were considered.

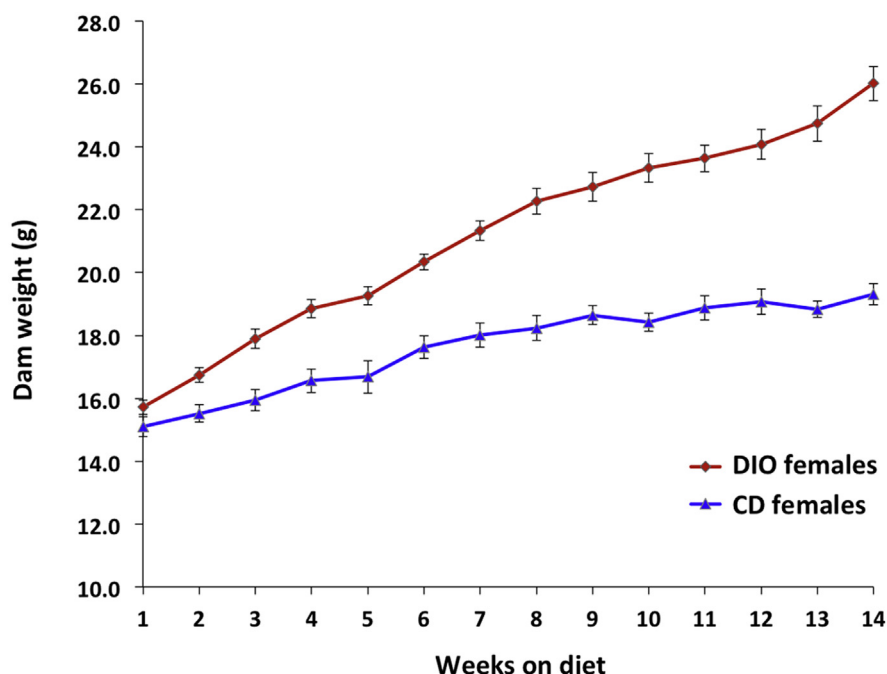
Whole-genome analysis of functional gene set regulation was determined by Gene Set Enrichment Analysis (GSEA),<sup>20</sup> using gene sets from the Developmental Functional Annotation at Tufts (DFLAT) database on fetal development (parameters as previously described).<sup>21</sup> Gene sets were considered significantly regulated if the FDR *q* value was  $< 0.20$ . Within each sex, analyses were performed for the following diet group comparisons: HFD/HFD vs CD; HFD/CD vs CD; HFD/CD vs HFD/HFD.

## Results

### Maternal DIO mouse model

Dam weight trajectories prior to pregnancy by diet group are depicted in Figure 1. Of 22 dams fed the HFD, 18 became obese after 14 weeks of feeding. Four dams did not meet criteria for obesity and were excluded. HFD-fed dams had significantly higher

**FIGURE 1**  
Dam weight trajectories prior to pregnancy



Diet-induced obese females were significantly heavier than their control diet counterparts at the end of the prebreeding feeding period.

CD, control diet; DIO, diet-induced obesity.

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percentage body fat and significantly lower percentage lean body weight than control females or males by EchoMRI analysis (Figure 2). Descriptive data for the dams and embryos by sex and diet group are reported in the Table. There were no significant differences between study groups with respect to litter size. There were significant differences between groups with respect to dam weight gain in pregnancy. Control dams gained the most weight in pregnancy, whereas the HFD/CD dams gained the least (Figure 3).

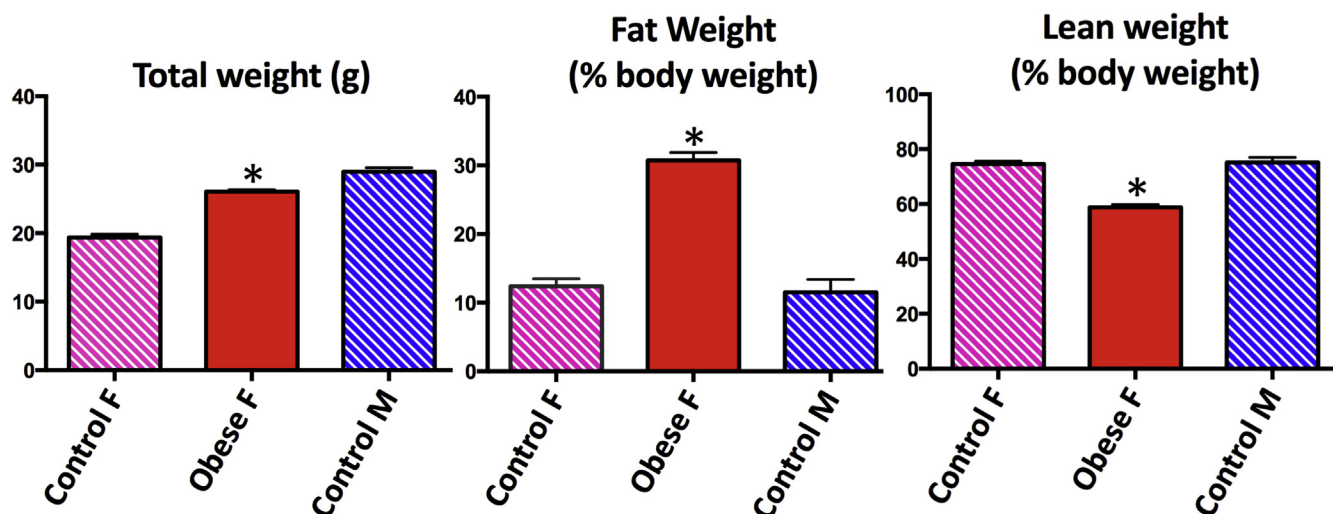
### Sex-specific differences in embryo size

Male embryos exposed to maternal obesity were significantly (approximately 17%) smaller in weight than their corresponding controls (Table;  $P = .01$ ). Female embryos demonstrated milder embryo size reduction in the setting of maternal obesity, which did not achieve statistical significance (9.5%,  $P = .26$ ).

### Sex-specific differences in embryonic brain gene expression profiles

Maternal obesity and maternal obesity with dietary change in pregnancy (herein

**FIGURE 2**  
Adult body composition prior to breeding



Diet-induced obese females had a significantly higher percentage body fat and lower percentage lean body weight compared with control females and males.

F, female; M, male.

\*Statistically significant.

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TABLE

## Dam and embryo characteristics by sex and diet group

Characteristic	Sex	Diet group			P value <sup>a</sup>
		HFD/HFD	CD/CD	HFD/CD	
Embryo weight, g (mean ± SEM)	Male	0.85 (0.04)	1.02 (0.04)	0.95 (0.04)	.01
	Female	0.86 (0.04)	0.95 (0.04)	0.88 (0.03)	.26
	Combined	0.85 (0.03)	0.98 (0.03)	0.91 (0.03)	.007
Embryo length, mm (mean ± SEM)	Male	19.58 (0.36)	21.00 (0.31)	21.02 (0.42)	.02
	Female	19.49 (0.36)	20.53 (0.35)	19.87 (0.35)	.28
	Combined	19.54 (0.25)	20.78 (0.23)	20.37 (0.28)	.01
Dam weight at breeding, g (mean ± SEM)	N/A	28.63 (0.64)	20.32 (0.35)	28.03 (0.99)	.002
Dam weight gain in pregnancy, g (mean ± SEM)	N/A	13.18 (2.2)	15.68 (0.96)	8.57 (1.35)	.01
Litter size, n (mean ± SEM)	N/A	7.25 (1.03)	7.83 (0.40)	6.33 (1.12)	.44

HFD/HFD, high-fat diet/high-fat diet; CD, control diet; HFD/CD, high-fat diet/control diet.

<sup>a</sup> P value was determined by Kruskal-Wallis testing.

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after referred to as dietary change) resulted in more dysregulated genes in male compared with female fetal brains (386 in males vs 66 in females,  $P < .001$ ). These numbers reflect the total number of unique DEGs in males and females for each of the diet group comparisons,

including HFD/HFD vs CD, HFD/CD vs CD, and HFD/HFD vs HFD/CD.

To consider only those genes differentially regulated by maternal obesity with or without dietary change in pregnancy, we determined the sum of the HFD/HFD vs CD and HFD/CD vs CD

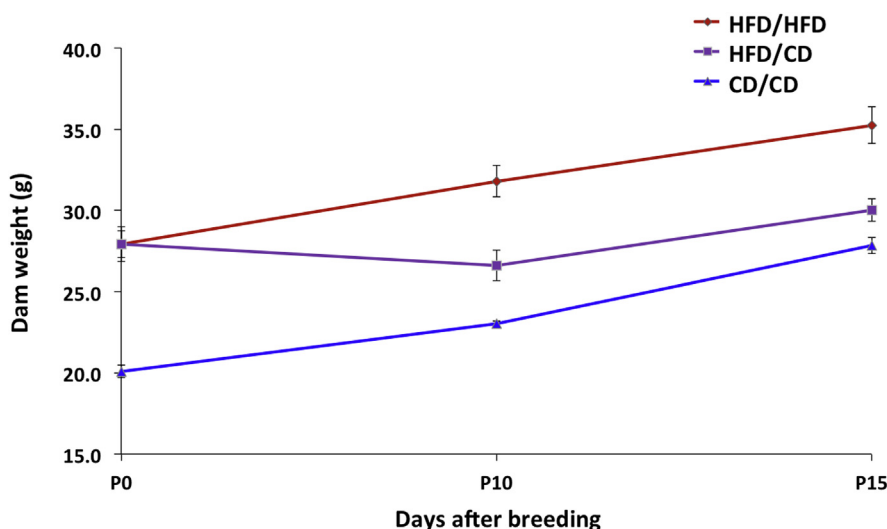
comparison, again finding a significant difference between the sexes in the number of dysregulated genes (223 in males vs 28 in females,  $P < .001$ ).

Figures 4, A–D, depict Venn diagrams for the number of unique and common differentially expressed genes in males vs females for each comparison of maternal obesity and dietary change in pregnancy. Maternal obesity and dietary change are associated with distinct brain gene expression signatures for each sex, with overlap of only 1 gene (*nuclear encoded rRNA 5S 170*, function unknown) across all comparisons (Figure 4D). Maternal obesity with dietary change during pregnancy resulted in more DEGs in the fetal brain than continuation on the HFD. Supplemental Files 2 and 3 show the list of significant DEGs for the males and females for each diet group comparison.

Gene expression changes in males and females compared with their corresponding controls were further visualized as a heat map (Figure 5A). The hierarchical clustering and expression intensity confirm stronger gene expression changes in males v females in the setting of maternal obesity and dietary change. Within these DEGs, a block of 37 differentially expressed stem-loop microRNAs (miRNAs) were identified (Figure 5B). For these miRNAs, the

FIGURE 3

## Pregnancy weight trajectories by diet group



HFD/HFD and CD/CD dams gained weight steadily throughout pregnancy. HFD/CD dams initially lost weight at P10 but by P15 had gained an average of 2.1 g over their prepregnancy weight.

CD/CD, control diet before pregnancy and during pregnancy; HFD/CD, high-fat diet before pregnancy and control diet during pregnancy; HFD/HFD, high-fat diet before pregnancy and during pregnancy; P, postnatal day.

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stronger effects of maternal obesity and dietary change on male fetuses are even more pronounced.

A principal component analysis was performed to identify the dominant sources of variation in the gene expression data. The most pronounced clustering was by sex (Figure 6). Subtle clustering by diet group was observed in the male but not the female brain gene expression data. There were no significant differences in within-group variability between males and females or between any of the diet groups.

The average relative variation in gene expression for each diet group was as follows: female CD/CD, 14.9%; female HFD/CD, 15.3%; female HFD/HFD, 17.4%; male CD/CD, 15.9%; male HFD/CD, 14.9%; and male HFD/HFD, 14.9%. Principal component analysis also demonstrated that gene expression in the samples cannot be distinguished by litter within the male or female diet groups (ie, no evidence of litter effects).

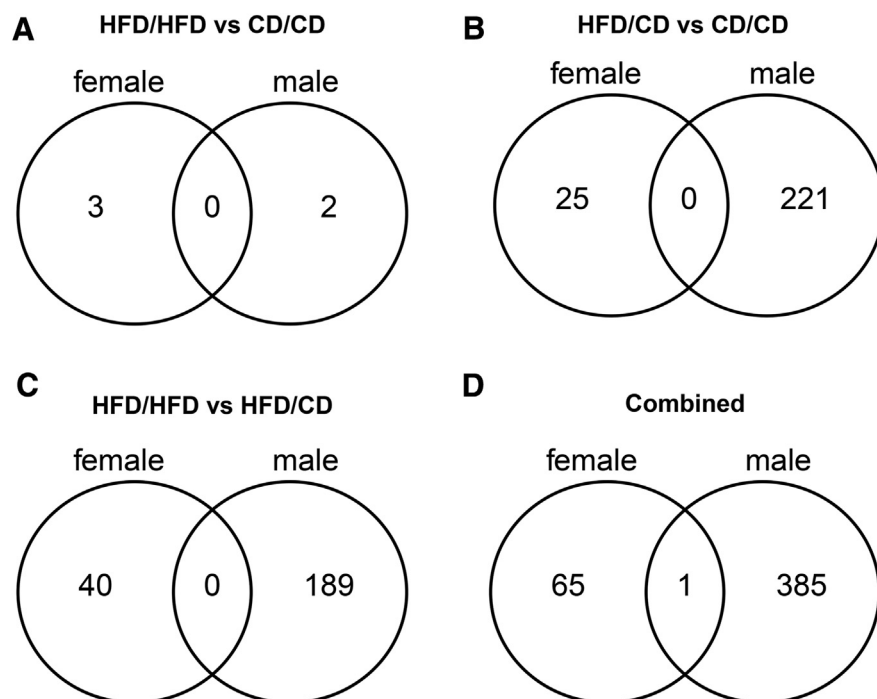
### Pathway analyses

Pathway analyses were performed using IPA and GSEA/DFLAT. The working files for the IPA analyses may be found in [Supplemental File 4](#). Dysregulated biological processes and pathways in males, females, and both are depicted for each diet group comparison in [Figures 7-9](#).

Pathway analyses demonstrated that male and female fetal brains had several dysregulated processes in common across all 3 comparisons (immune/inflammatory signaling, cell cycle regulation, insulin signaling, and vascular pathology). Biological processes that were specifically dysregulated in females included iron homeostasis, leptin signaling, and angiogenesis.

Pathways that were dysregulated specifically in males included those implicated in actomyosin contractility, neuromuscular disorders, and connective tissue development. Males had significantly more dysregulated miRNAs (36 vs 1,  $P < .001$ ). These miRNAs have been implicated in proinflammatory and immune signaling, cell death, adipogenesis, and oxidative stress response among other functions. A description of

**FIGURE 4**  
Number of differentially regulated brain genes in females versus males in maternal obesity



**A–D**, Number of significantly differentially regulated brain genes in females vs males in maternal obesity with and without dietary change in pregnancy. Maternal obesity alone and with dietary change in pregnancy was associated with distinct brain gene expression signatures for females and males, with an overlap of only 1 gene across all comparisons.

CD/CD, control diet before pregnancy and during pregnancy; HFD/CD, high-fat diet before pregnancy and control diet during pregnancy; HFD/HFD, high-fat diet before pregnancy and during pregnancy.

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the stem-loop miRNAs, corresponding mature miRNAs, and putative dysregulated target genes and pathways appears in [Supplemental File 5](#).

Notably, different aspects of brain development were altered in males vs females for each of the 3 comparisons. Brain development terms implicated in both sexes included pathways related to developmental delay, cerebral cortex development, and neurotransmitter signaling. Brain development terms dysregulated in only females included negative regulation of neuronal and glial apoptosis, cranial nerve and dentate gyrus formation, forebrain regionalization, and axonogenesis. Terms dysregulated in males only included neurodegeneration, astrocyte differentiation, midbrain development, synaptic plasticity, neuro-peptide hormone signaling, visual

learning and memory, cued conditioning, and social behavior. Significantly dysregulated pathways and gene sets identified by IPA and GSEA/DFLAT for each comparison are presented in [Supplemental Files 6 and 7](#).

### Comment

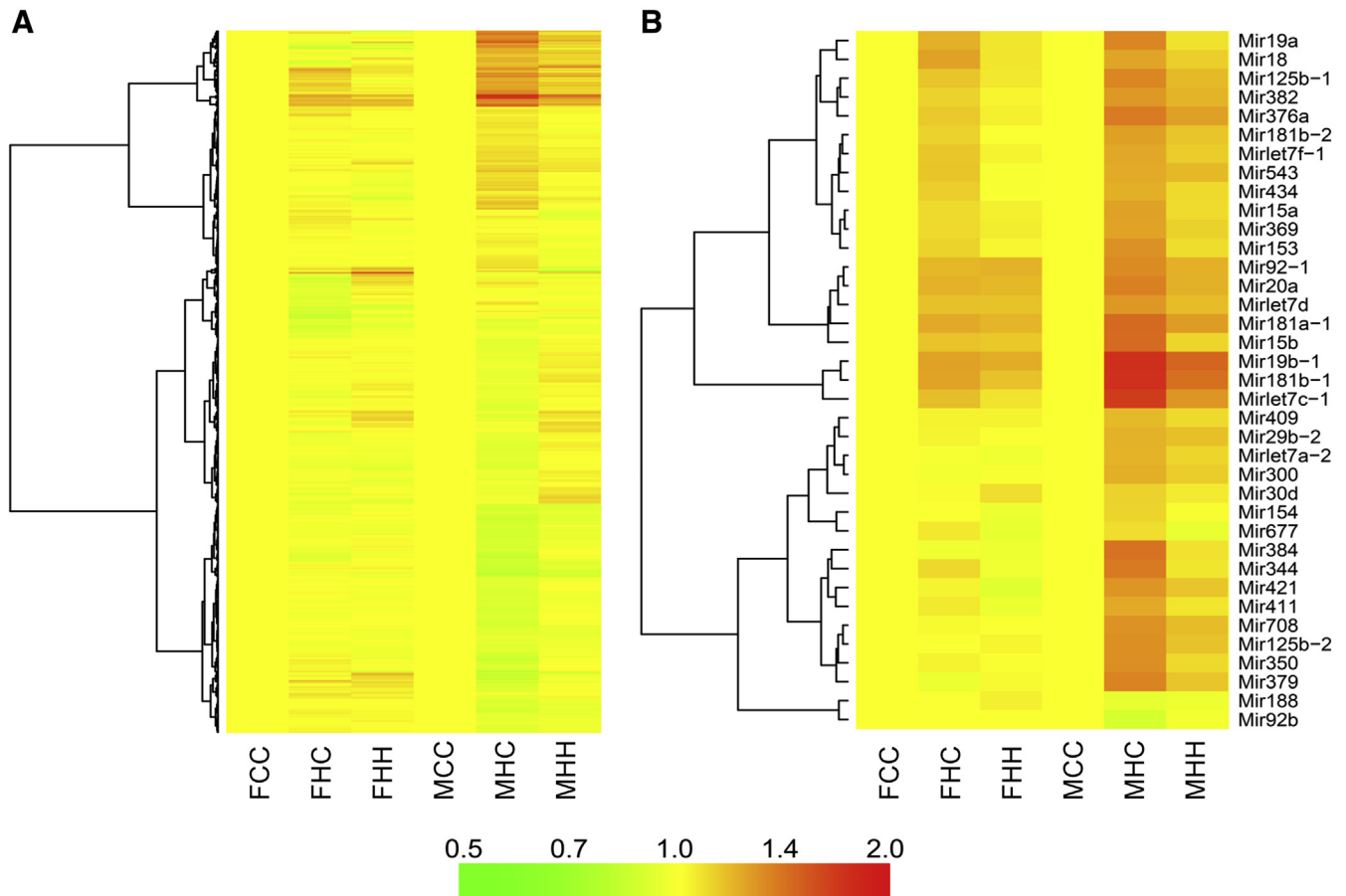
#### Principal findings

In this study, we demonstrated that maternal obesity is associated with statistically significant sex-specific differences in fetal size and fetal brain gene expression signatures. Male embryos exposed to maternal obesity and an HFD in utero were significantly smaller than controls, whereas weight differences were less pronounced for females.

In the setting of maternal obesity and dietary change, males and females had

FIGURE 5

Heat map of differentially regulated brain genes and microRNAs by sex and diet group



Heat map of differentially regulated brain genes (A) and microRNAs (B) by sex and diet group. Maternal obesity and dietary change in pregnancy had a stronger effect on male brain gene expression. Red indicates higher expression, green indicates lower expression, and yellow is neutral. Heat map values correspond to expression ratios relative to the matched control diet for that sex (1, unchanged; 2, 2-fold increased expression compared with controls; 0.5, 2-fold decreased expression compared with controls).

FCC, female control group; FHC, female high-fat diet before pregnancy and control diet during pregnancy; FHH, female high-fat diet before pregnancy and during pregnancy; MCC, male control group; MHC, male high-fat diet before pregnancy and control diet during pregnancy; MHF, male high-fat diet before pregnancy and during pregnancy.

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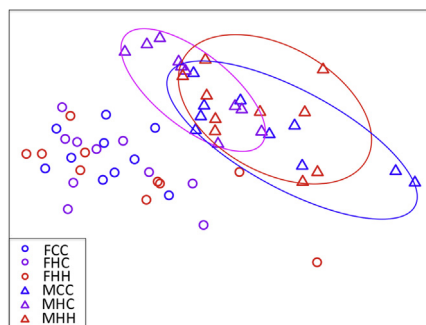
unique brain gene expression signatures with overlap of only 1 gene. More dysregulated genes and stronger differential regulation were noted in male compared with female brains. Pathway analyses demonstrated significant dysregulation of the cell cycle and of immune, inflammatory, and insulin signaling in the brains of both sexes and highlighted unique dysregulated aspects of brain development for each sex. Switching obese dams to a control diet in pregnancy had a stronger impact on embryonic brain gene expression than did continuing the HFD.

These data help fill a knowledge gap. Little is known regarding the mechanisms that underlie the association between maternal obesity and adverse neurodevelopmental outcomes for offspring and the relative contributions of the in utero vs postnatal environment. The use of a mouse model permitted direct examination of the fetal brain in obese pregnancy, which allowed us to definitively address possible in utero origins of the postnatal neurodevelopmental morbidities noted in epidemiological studies.

Few studies have examined sex effects of maternal obesity on fetal brain development and offspring behavior.<sup>8,11</sup> To our knowledge, this is the first study to examine the effects of maternal obesity on the fetal brain in both sexes. The limited number of studies that have investigated the effects of maternal obesity on brain development focused on the postnatal period,<sup>8,11,22</sup> on male embryos and pups only,<sup>22-24</sup> or the fetal sex was unknown/not considered.<sup>25</sup> A mechanistic understanding of how sex modifies the effect of maternal obesity on fetal brain development is critical

FIGURE 6

## Principal component analysis of significantly differentially regulated brain genes



Males are represented by triangles and females by circles. Gene expression segregates most strongly on the basis of fetal sex, with subtle clustering of gene expression by diet group noted in males but not females. On the x-axis is principal component 1, which accounts for 28.9% of the variance in gene expression data. On the y-axis is PC 2, which accounts for 7.8% of the variance.

FCC, female control group; FHC, female high-fat diet before pregnancy and control diet during pregnancy; FHH, female high-fat diet before pregnancy and during pregnancy; MCC, male control group; MHC, male high-fat diet before pregnancy and control diet during pregnancy; MHH, male high-fat diet before pregnancy and during pregnancy.

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to designing effective and targeted interventions.

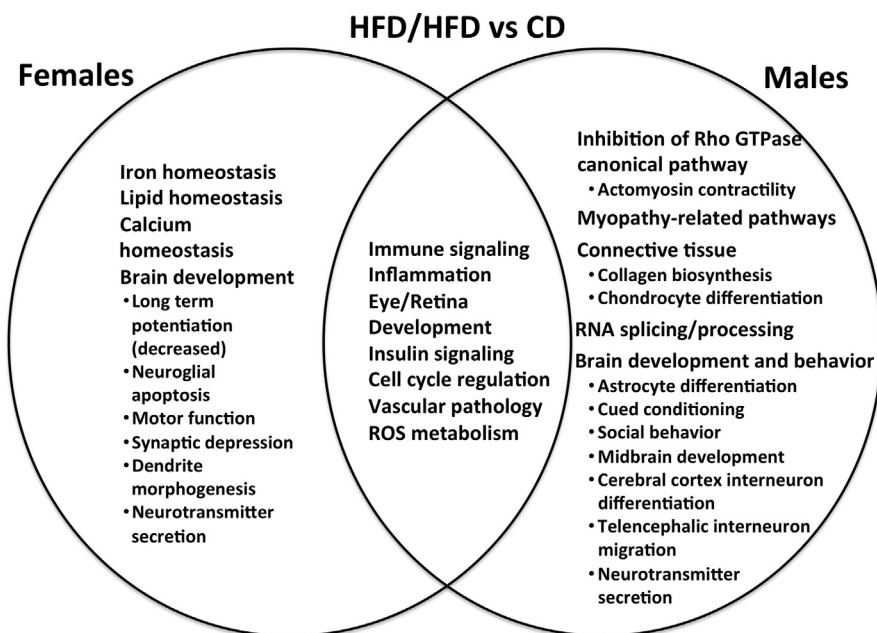
## Findings in context

The significant sex differences noted in our study, with very little overlap between DEGs in males and females, are plausible based on prior literature. The murine placental transcriptome in the setting of maternal HFD is sexually dimorphic, with very few dysregulated genes in common and marked differences between the sexes in the biological functions of dysregulated genes.<sup>26</sup>

One other study has examined the fetal brain transcriptome in a rat model of a maternal high-carbohydrate diet but had fewer subjects per group and did not consider fetal sex.<sup>25</sup> The few studies examining neurodevelopmental sex differences in the setting of maternal obesity have focused on the postnatal period.<sup>8,10,11</sup> In a similar murine DIO model with a shorter period of

FIGURE 7

## Common and unique dysregulated pathways in the female and male embryonic brain in maternal obesity



This Venn diagram compares common and unique pathways dysregulated in male and female brains exposed to maternal obesity and a high-fat diet in utero (HFD/HFD group) against a reference group of male or female controls (CD). Males and females were each compared with their corresponding controls. Pathways reflect results from both Ingenuity Pathways Analysis and Gene Set Enrichment Analysis with Developmental Functional Annotation at Tufts databases.

CD/CD, control diet before pregnancy and during pregnancy; GTPase, guanosine 5'-triphosphate; HFD/CD, high-fat diet before pregnancy and control diet during pregnancy; HFD/HFD, high-fat diet before pregnancy and during pregnancy; RNA, ribonucleic acid; ROS, reactive oxygen species.

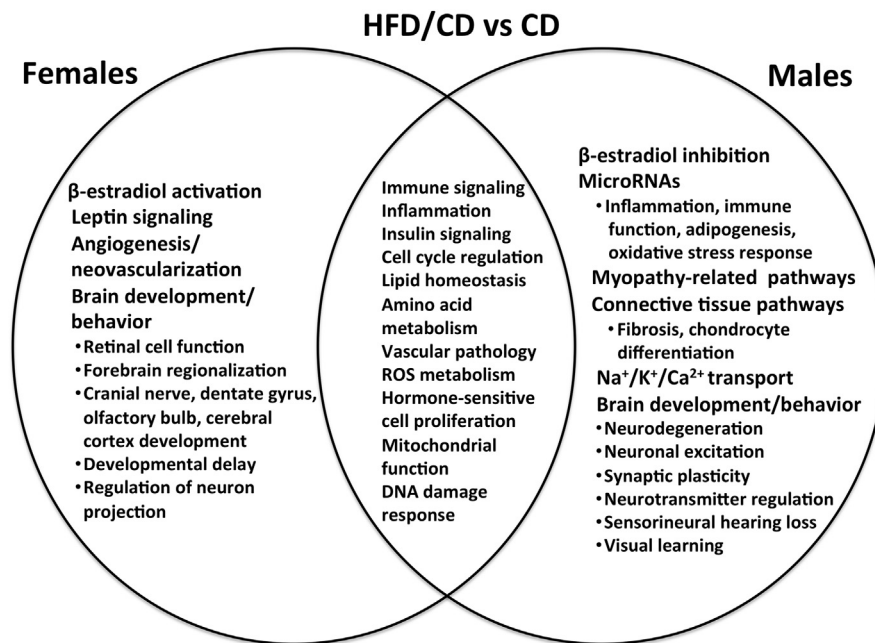
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prebreeding feeding, exposure to a maternal HFD during gestation and lactation resulted in increased hyperactivity in male offspring and increased anxiety and decreased sociability in female offspring.<sup>8</sup> This model suggested higher postnatal sensitivity of female pups to maternal dietary change in lactation, reporting amelioration of female, but not male, neurobehavioral phenotype and neuroinflammation after switching dams to a control diet during lactation. In a rat model of maternal HFD, male offspring displayed increased anxiety but improved spatial learning compared with female offspring and controls.<sup>11</sup> There appear to be sex-specific effects of maternal obesity and maternal diet on fetal and offspring neurodevelopment, but it is not clear whether one sex is more adversely affected.

The finding that male fetal growth and brain gene expression may be more sensitive to environmental influences during pregnancy is also consistent with prior studies. Males have traditionally been viewed as more vulnerable to gestational and perinatal exposures compared with females.<sup>27,28</sup> There is a higher frequency of male fetuses miscarried and stillborn,<sup>29</sup> a lower rate of neonatal survival in males compared with females at a given gestational age,<sup>30</sup> and an increased incidence of neurological and nonneurological perinatal complications in males.<sup>31</sup> In addition, males are at increased risk for neurodevelopmental morbidity compared with females born at the same gestational age.<sup>31</sup>

A variety of hypotheses have been proposed to explain increased male neurodevelopmental vulnerability,

FIGURE 8

**Common and unique dysregulated pathways in the female and male embryonic brain in maternal obesity with dietary change in pregnancy**

This Venn diagram compares common and unique pathways dysregulated in male and female brains exposed to maternal obesity and a control diet in utero (HFD/CD group) against a reference group of male or female controls (CD). Males and females were each compared with their corresponding controls. Pathways reflect results from both ingenuity pathways analysis and Gene Set Enrichment Analysis with Developmental Functional Annotation at Tufts database.

CD, control diet; DNA, deoxyribonucleic acid; HFD/CD, high-fat diet before pregnancy and control diet during pregnancy; RNA, ribonucleic acid; ROS, reactive oxygen species.

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including delayed male brain maturation in utero,<sup>27</sup> androgen exacerbation of excitotoxic cell death,<sup>32</sup> and sex differences in cell death pathways that may favor females.<sup>28</sup> With respect to specific pathways and processes implicated in our data, maternal obesity is known to be an inflammatory state, and we identified gene expression patterns consistent with increased inflammation in both male and female fetal brains. The male cerebral cortex may be more vulnerable to inflammation in utero; male cortical astrocytes were noted to produce more proinflammatory mediators after exposure to lipopolysaccharide than did female astrocytes, an effect that appeared to be mediated in part by testosterone exposure.<sup>33</sup>

Sex differences in microglial function and subsequent neuroinflammation during development have also been

proposed as a mechanism by which males are predisposed to later neurological and neurodegenerative disorders.<sup>34</sup> In a mouse model of intrauterine inflammation, increased male brain vulnerability was described, with male adult offspring more likely to have hippocampal volume loss on magnetic resonance imaging and cortical infiltrating monocytes on histological examination.<sup>35</sup> Further investigations are needed, however, to determine whether the increased number of DEGs in the male brain is associated with more neurodevelopmental morbidity (see *Clinical and Research Implications*).

### Clinical and research implications

Additional research is required before the information presented here can be translated to a human clinical setting. First, it is not clear whether the

differences noted in fetal brain gene expression will translate to functional differences in offspring neurobehavior, so we plan neurobehavioral testing on offspring of both sexes from the 3 diet groups. Second, the human correlate of the profound effect of obese females changing to a control diet in pregnancy is unknown. It may be better if obese women undertake significant dietary and lifestyle changes prior to, rather than during, pregnancy.

### Strengths and limitations

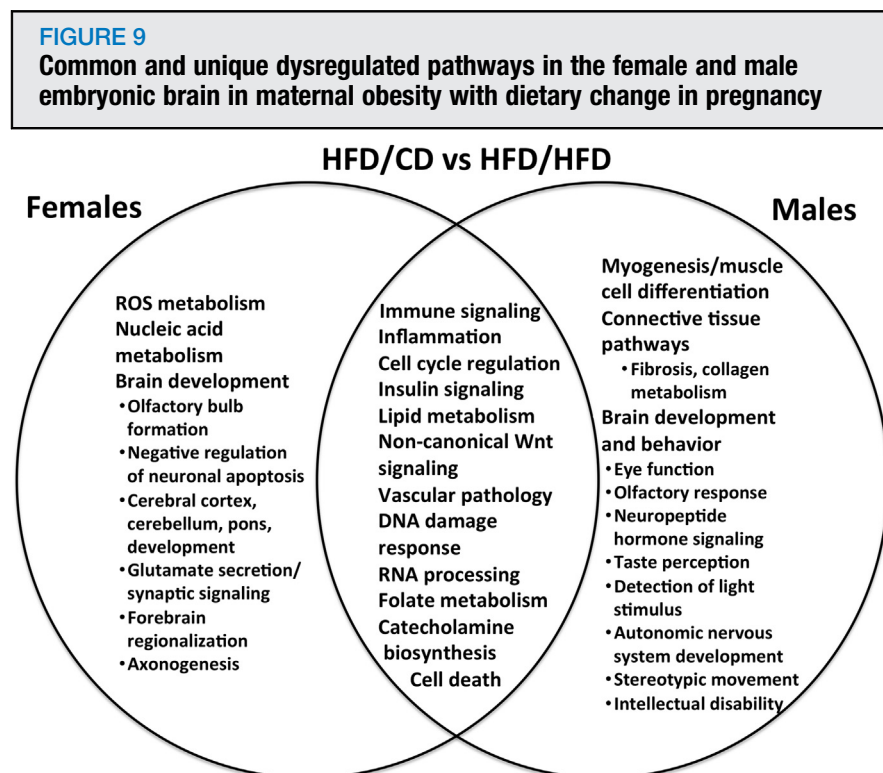
Strengths of our study include the whole-transcriptome approach, which does not preselect for specific gene transcripts or pathways based on prior knowledge. Another strength is our comprehensive functional analysis with multiple bioinformatics resources, including a resource annotated specifically for the fetus (DFLAT).<sup>21</sup> Prior work has demonstrated the importance of using more than 1 systems biology resource for the most complete understanding of dysregulated biological processes.<sup>36</sup> The weight differences between the obese and lean dams prior to breeding are more significant in our study than in most other studies that have used a DIO model to examine the effects on offspring neurodevelopment.<sup>8,11,22,24</sup> Ensuring substantial weight differences between the HFD-fed and CD-fed dams allowed us to examine the effects of true maternal prepregnancy obesity and not just maternal HFD on fetal brain development. Finally, our examination of the fetal effects of maternal obesity separately from a maternal HFD in pregnancy has not been performed in other studies and permitted the detection of the significant effect of dietary change in pregnancy on fetal brain gene expression.

As a limitation, the study was designed with 3 diet groups to isolate prepregnancy maternal obesity as the variable influencing fetal brain gene expression in the HFD/CD group. We did not envision the HFD/CD group as a dietary intervention group. The unexpected finding that obese females eating a control diet in pregnancy was associated with more



differentially expressed fetal brain genes than maternal obesity and continuation on a HFD suggests that the change to a CD in pregnancy might represent more of an intervention than a return to the baseline situation. This is a limitation because we are not able to tease apart the relative contributions of maternal obesity and the dietary switch on fetal brain gene expression. The 2 variables may act synergistically to have an impact on fetal brain gene expression, or there may be a third unmeasured effect (such as maternal stress) that is driving the changes. The fact that both males and females exposed to dietary change had annotations related to dysregulated catecholamine compound biosynthesis (including the neurotransmitters adrenaline, noradrenaline, and dopamine) may suggest that maternal/fetal stress could be playing a role in dysregulated brain gene expression. The HFD/CD dams did not appear unhealthy or malnourished during their pregnancy. Although they lost an average of 1.3 g by P10 of their pregnancy, they gained an average of 2.1 g by P15, suggesting the dietary change was not consistent with starvation-type conditions. Formal behavioral or biochemical evaluations of maternal stress after switching to the control diet in pregnancy were not performed because these findings were not anticipated. Whether the significant fetal effects of dietary change in pregnancy for the obese dams (or the relatively few dysregulated genes in the setting of continuation of maternal HFD) have a direct human parallel is unclear. Even so, these data are important because they highlight the key consideration of maternal pregnancy diet as a separate variable in studies such as these.

The relatively small fold-change differences between groups might be perceived as a limitation. Although the effect size of the fetal brain gene expression differences because of maternal obesity and maternal dietary change in pregnancy are smaller than one would expect to find in gene expression studies of conditions such as cancer or infection, these modest effect sizes are not only biologically plausible but also could



This Venn diagram compares common and unique pathways dysregulated in male and female brains exposed to maternal obesity and a control diet in utero (HFD/CD group) against a reference group of males or females exposed to maternal obesity and a high-fat diet in utero (HFD/HFD group). Pathways reflect results from both ingenuity pathway analysis and Gene Set Enrichment Analysis with Developmental Functional Annotation at Tufts database.

DNA, deoxyribonucleic acid; HFD/CD, high-fat diet before pregnancy and control diet during pregnancy; HFD/HFD, high-fat diet before pregnancy and during pregnancy; RNA, ribonucleic acid; ROS, reactive oxygen species.

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result in a distinct neurobehavioral phenotype. A recent study of fetal brain gene expression in a mouse model of Down syndrome found similarly modest fold changes and an abnormal neurobehavioral phenotype in pups.<sup>37</sup>

## Conclusions

Maternal obesity and dietary change in pregnancy are associated with distinct fetal brain gene expression signatures for males and females and more dysregulated genes in the male brain. Pathway analysis suggests different aspects of brain development are dysregulated in males vs females. Male growth and male brain gene expression may be more sensitive to environmental influences during pregnancy, such as maternal obesity and maternal diet. It is important to consider both fetal sex and maternal diet when evaluating

the effects of maternal obesity on neurodevelopment. ■

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