Maternal obesity alters fetal neuroinflammation in a murine model of preterm birth

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BACKGROUND: Preterm birth from intrauterine infection is a leading cause of neonatal neurologic morbidity. Likewise, maternal obesity is associated with intra-amniotic infection and inflammation. Whether maternal obesity is a risk factor for fetal brain injury that occurs with premature birth remains unknown. This study hypothesized that maternal obesity intensifies fetal neuroinflammation in the setting of premature delivery.
OBJECTIVE: This study aimed to examine the influence of maternal obesity on perinatal neuroinflammatory responses that arise with preterm birth using a murine model.
STUDY DESIGN: Dams with obesity were generated via a high-fat diet that was maintained throughout pregnancy. In parallel, dams without obesity (normal) received a control diet. All dams were paired with males on normal diet. Pregnant dams were randomized to receive an intrauterine administration of bacterial endotoxin (lipopolysaccharide) or the vehicle (phosphate-buffered saline) on embryo day 15.5 of what is typically a 19- to 21-day gestation. Fetal brains were harvested 6 hours after intrauterine administrations, and the expressions of key inflammatory cytokines (*Il1b*, *Il6*, and *Tnf*) and panels of metabolic, immune, and inflammatory genes were analyzed.
RESULTS: With the phosphate-buffered saline, there was no difference in gene expression related to maternal obesity. There were substantial differences in *Il6* and immune/inflammatory expression profiles in fetal brains from dams with obesity vs normal dams that received lipopolysaccharide. Few differences were observed among the metabolic genes examined under these conditions. The gene expression pattern associated with maternal obesity correlated with pathways related to white matter injury.
CONCLUSION: The expression of neuroinflammatory markers instigated by bacterial endotoxin via intrauterine lipopolysaccharide was greater in embryo brains obtained from dams with obesity. Expression profiles suggest that in combination with intrauterine inflammation, maternal obesity may increase the risk of fetal white matter injury. Further investigation is warranted to understand the relationship between maternal health and neurologic outcomes associated with prematurity.

Key words: fetal brain, intrauterine infection, interleukin, maternal obesity, neuroinflammation, neurodevelopment, pregnancy, premature birth, white matter injury, mouse model

Introduction

Preterm birth poses significant risks to fetal brain development. One of the strongest risk factors for preterm birth and developmental disability is intra-amniotic inflammation, commonly mediated by bacteria.[1–4] Inflammatory responses to infection are believed to be responsible for neurodevelopmental impairment, with greater incidence and severity at earlier gestational ages of premature birth.[5] As the incidence of premature birth has increased across the United States, so has the prevalence of maternal obesity.[6] Obesity is associated with an increased risk of adverse obstetrical complications, including intra-amniotic infection, preterm birth, and neonatal neurologic morbidity.[7,8] Chronic low-grade inflammation is common among individuals with obesity and may promote delivery and developmental complications via the systemic release of proinflammatory cytokines.[9] Excessive adipose tissue secretes proinflammatory factors, such as interleukin 1 beta (IL-1B), interleukin 6 (IL-6), and tumor necrosis factor (TNF), that are linked to premature birth and perinatal brain injury mainly to white matter.[10–12] Chronic inflammation by these cytokines in pregnancies complicated by obesity may

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AJOG MFM at a Glance

Why was this study conducted?

This study was conducted to determine whether there is a relationship between obesity and neuroinflammation in a lipopolysaccharide murine model of preterm labor.

Key findings

The key findings included an association between markers of inflammation, including interleukin 6 and up-regulated inflammatory gene expression.

What does this add to what is known?

Obesity is associated with inflammation in many disease states; however, its association with neuroinflammation in cases of preterm labor is not well elucidated. This study adds to the literature the fact that obesity is also associated with neuroinflammation in an animal model of preterm labor.

increase the risk of neurodevelopmental consequences observed with premature birth.[13–16]

Although obesity and preterm birth are linked, it remains unclear whether immunologic responses to intra-amniotic infection change with maternal obesity. In humans, the effect of obesity is difficult to discern from the characteristics associated with neuroinflammatory injury, which include gestational age at delivery, etiology of infection, genetics, and fetal sex.[17] To control for such variables, we examined the influence of maternal obesity in a murine model of intrauterine inflammation and preterm birth.[18–21] In this model, the key inflammatory cytokines *Il1b*, *Il6*, and *Tnf* were elevated, reflecting observations in human amniotic fluid with premature birth.[22] We hypothesized that maternal obesity compounds fetal immune responses that can negatively affect neurodevelopment. The primary objective of this study was to examine whether neuroinflammation stimulated by bacterial lipopolysaccharide (LPS), intensifies in the progeny of dams with obesity.

Materials and methods

Animal experiments and procedures were performed under the guidance and approval of the Institutional Animal Care and Use Committee. Female C57BL/6J without (normal) and with diet inducted obesity were acquired from Envigo. Mice in the cohort of obesity were provided a 60% high-fat diet ad lib consistently beginning at 3 weeks of age, the time of weaning (Teklad TD.06414). Mice were received obese and maintained on the same high-fat diet until euthanasia. In parallel, normal female mice received a comparable control diet consisting of 10% fat (Teklad TD.08806). All dams were mated with male C57BL/6J also from Envigo, given a normal diet to eliminate paternal obesity as a potential confounder. Following the preterm murine modality established by Elovitz et al,[21] all pregnant dams were randomized to receive an intrauterine administration of LPS from *Escherichia coli* (O127.B8 from Sigma-Aldrich, St. Louis, MO) or phosphate-buffered saline (PBS) on day 15.5 (E15.5).[23] The dams were anesthetized with isoflurane, and a mini-laparotomy was performed to access the uterine horns to administer 250 μg LPS in 100 μL PBS or the equivalent volume of the vehicle (Figure 1, A). Embryos were harvested 6 hours after intrauterine administration and either fixed whole in formalin or their brains were dissected and flash frozen in liquid nitrogen. This timepoint was chosen on the basis of the studies by Gayle et al[24] and Brown et al,[25] which show the up-regulation of inflammatory cytokines before delivery and loss of viability that can result beyond 6 hours of LPS exposure. Fixed whole embryos were weighed within 7 days of collection.[27] Embryo brains were used for RNA isolation and subsequent gene expression analyses as previously described using targeted gene expression panels intended to provide insight into neuroinflammatory responses to LPS, particularly the molecular pattern of microglia covered by the NanoString neuroinflammatory panel.[23,26–28] The same samples were randomly selected and used for each analysis. Briefly, for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of individual genes and panels, fold changes were calculated from relative quantifications normalized to ribosomal *18s* using the ΔΔCt method.[29] Tables and graphs were generated using Microsoft Excel, and statistical analyses for polymerase chain reaction (PCR) gene expression were performed using IBM SPSS software (version 28; IBM, Armonk, NY). The analysis of differentially expressed genes (DEGs) from the NanoString neuroinflammatory panel was performed using the nSolver Advanced Analysis software (version 4.0; NanoString Technologies Inc, Seattle, WA). From this analysis, statistically significant DEGs (those below an adjusted $P<.05$) were matched to canonical pathways using the Qiagen ingenuity pathway analysis (IPA). To remove poorly associated processes, IPA canonical pathways with a $-\log(P$ value) of <7 were filtered from this analysis. The IPA analysis is presented as a bubble chart sorted by z scores along the y-axis and P values along the x-axis. A complete list of genes and results for each expression panel and IPA analysis is listed in the Supplemental methods and data file.

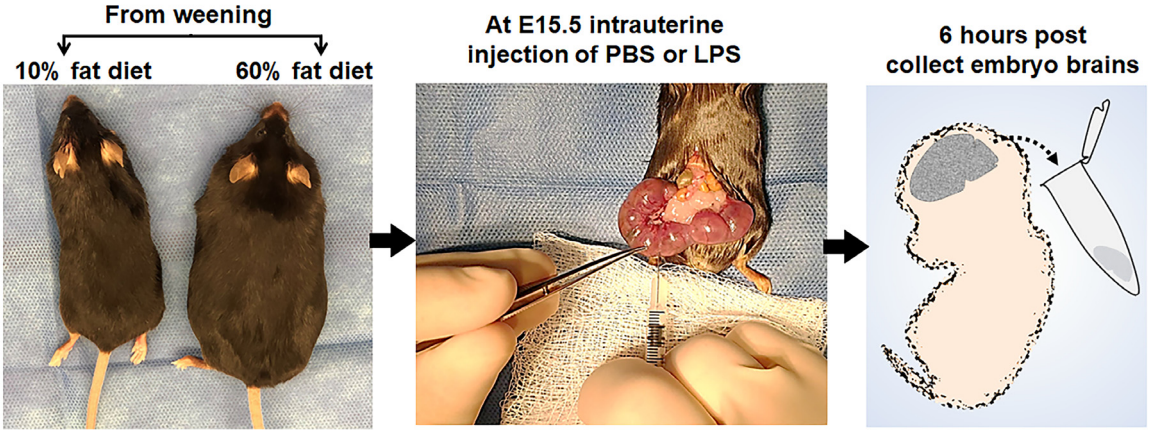
Results

A significant difference in maternal weight at conception and at the time of embryo collection was observed between obese and normal dams (Figure 1, B). Other characteristics, such as number of embryos and their weight, were similar between dams.

Analysis of key inflammatory genes shows significant up-regulation with LPS exposure (Figure 2, A). The expression of *Il6* was significantly greater in the fetal brains of dams with obesity than in the fetal brains of normal dams. No difference was observed in PBS controls. Gene expression analysis

FIGURE 1
Experimental design and pregnancy characteristics

A Experimental design



B Pregnancy and fetal characteristics

Mean (±SEM)	Normal dams n=9	Obese dams n=10	% Difference	p-value
Duration of diet (weeks)	15.1 (±1.2)	15.7 (±1.2)	4%	0.695
Maternal age at conception (weeks)	18.1 (±1.2)	18.7 (±1.2)	3%	0.695
Maternal weight at conception (grams)	23.4 (±0.7)	29.6 (±1.6)	21%	***0.003***
Maternal weight at E15.5 (grams)	30.1 (±1.5)	37.1 (±1.9)	19%	***0.009***
Number offspring per dam	7.1 (±0.9)	7.3 (±0.6)	3%	0.860
Embryo weight (grams)	0.47 (±0.02)	0.46 (±0.02)	3%	0.920

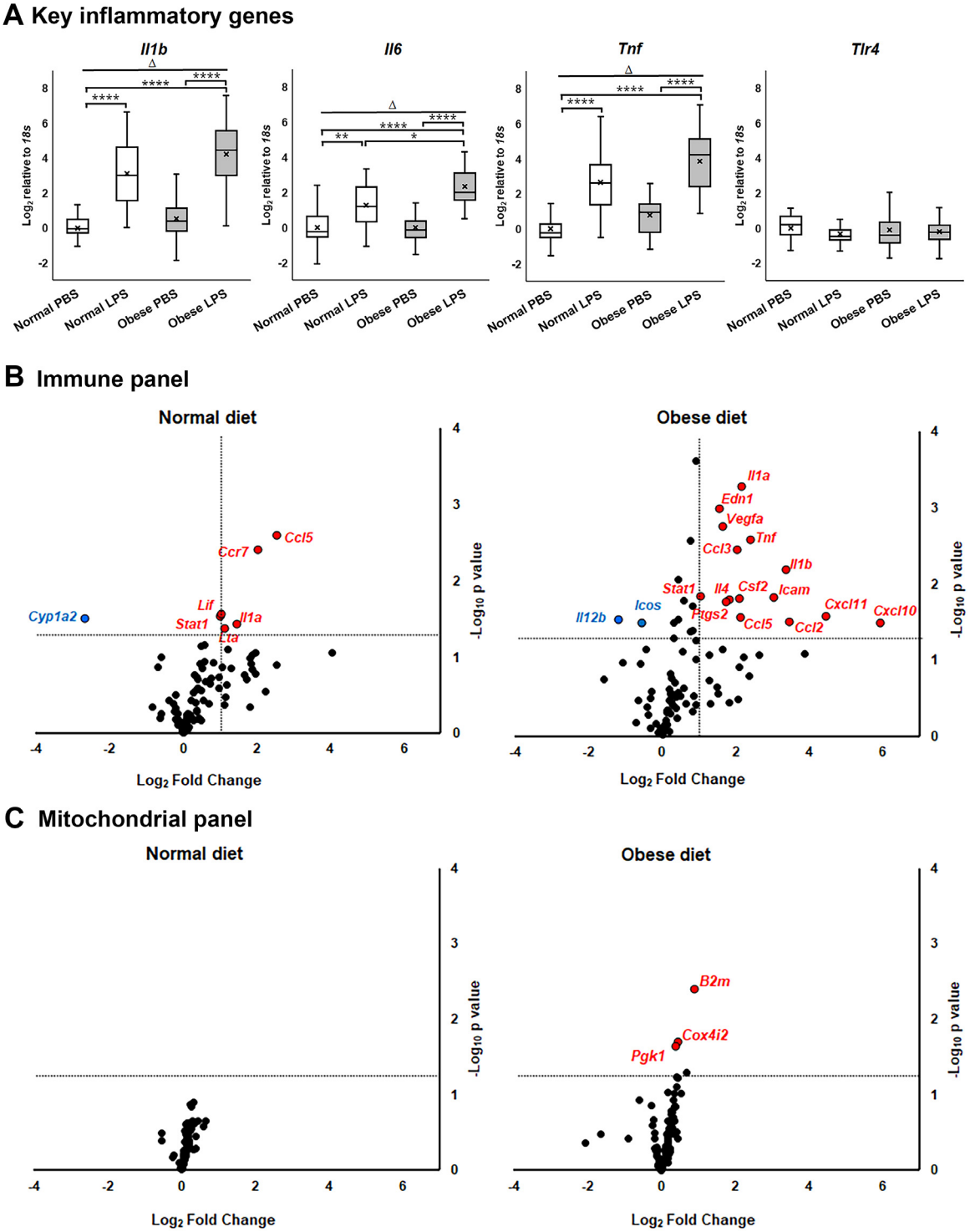
A, Outline of the experimental design specifying the timeline of diet introduction, intrauterine administrations, and subsequent fetal brain harvest. **B,** Maternal and fetal characteristics between normal dams and dams with obesity. *P* values were generated using the Student *t* test.

Leonard. Increased fetal neuroinflammation with maternal obesity in a murine model of preterm labor. Am J Obstet Gynecol Glob Rep 2024.

examining a panel of 92 immune genes across a smaller sampling of brains revealed substantial differences (Figure 2, B). Although this analysis was underpowered for detecting differences with LPS for key inflammatory genes shown in Figure 2, A, it is intended to provide a broader survey of differences between diet conditions. With LPS exposure, 6 genes were significantly up-regulated with a normal diet, whereas 26 genes were different with an obese diet. In contrast, a panel of 83 metabolic-related genes showed few differences within brains from embryos of dams with obesity compared with brains from embryos of normal dams (Figure 2, C). Specifically, no gene was different between LPS and PBS for the normal diet group, whereas only 3 genes were slightly up-regulated in the obese diet group.

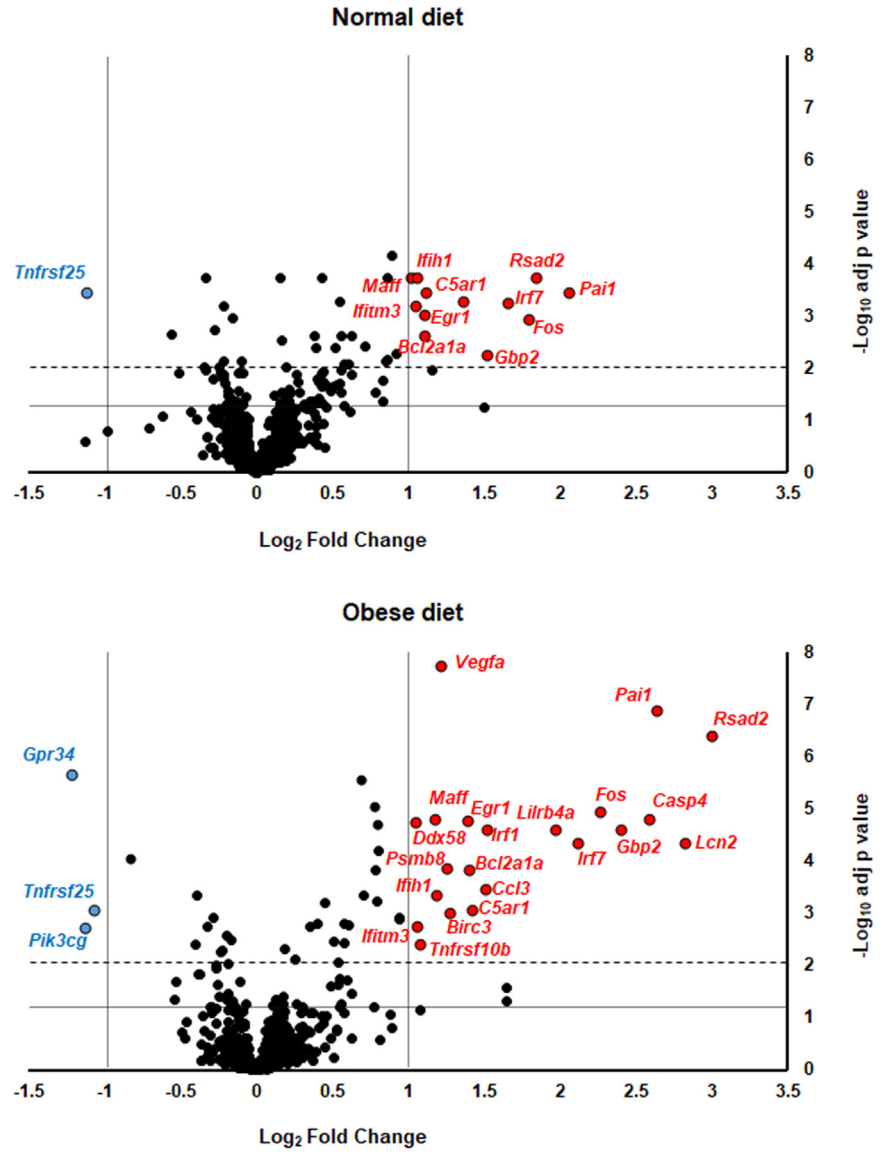
The analysis with a NanoString panel of 757 neuroinflammatory-associated genes yielded 594 DEGs between LPS and PBS for normal diet and 600 DEGs between LPS and PBS for obese diet (Figure 3 and Supplemental methods and data). Among the 594 DEGs detected for normal diet, 98 were below our criteria for statistical significance (adjusted $P<.05$). With the obese diet, 76 DEGs were statistically significant among the 600 DEGs detected. With a normal diet, 13 of 98 statistically significant DEGs had a fold change of >1, whereas 24 DEGs crossed this threshold, and the adjusted *P* values were generally lower with the obese diet. Among these genes, *Casp4* and *Lcn2* were up-regulated only with the obese diet (Figure 3), and *Spp1* was down-

FIGURE 2
Fetal brain immune and metabolic gene expression



A, Box and whisker plots show real-time PCR results for key inflammatory genes *Il1b*, *Il6*, *Tnf*, and *Tlr4*. Brains analyzed for normal PBS (n=16), normal LPS (n=21), obese PBS (n=23), and obese LPS (n=20). The relative expression normalized to *18s* is shown as $\log_2$ along the y-axis. The *x* within each box reflects the mean. The Δ reflects a $P<.05$ by 1-way analysis of variance, and the ** reflects a $P<.005$ and the **** reflects a $P<.00005$ by the Tukey posthoc test. **B and C,** Volcano plots for real-time PCR analysis using preformatted panels for 92 immune-related genes (**A**) and 83 mitochondrial-related genes (**B**) in 4 to 5 brains per condition. The differential expression between LPS vs PBS exposures normalized to *18s* is shown as $\log_2$ along the x-axis. *P* values are shown along the y-axis in $\log_{10}$. For normal diet, all genes with $P<.05$ are annotated *red* if significantly up-regulated and

FIGURE 3
Neuroinflammatory expression profile



Volcano plots show the differential expression for 757 DEGs within the NanoString neuroinflammatory panel from 15 fetal brains per condition. Expression shown as $\log_2$ along the x-axis reflects the brains from embryos exposed to LPS vs PBS, from normal dams **(A)** and dams with obesity **(B)**. False discovery rate adjusted *P* values are shown in $\log_{10}$ along the y-axis. DEGs with an adjusted *P*<.05 and a fold change of >1 are annotated *red* if up-regulated and *blue* if down-regulated. The *dotted horizontal lines* denote an adjusted *P*=.05, the *dashed lines* denote an adjusted *P*=.01, and the *dotted vertical lines* reflect a fold change of 1.

DEG, differentially expressed gene; *LPS*, lipopolysaccharide; *PBS*, phosphate-buffered solution

Leonard. Increased fetal neuroinflammation with maternal obesity in a murine model of preterm labor. Am J Obstet Gynecol Glob Rep 2024.

regulated with the normal diet but up-regulated with the obese diet (Supplemental methods and data).

The expression pattern among genes that were statistically significant (adjusted *P*<.05) (Supplemental methods and data) from the NanoString panel linked to similar IPA canonical pathways among normal and obese diets (Figure 4). Similar patterns and levels of statistical significance were observed across 43 IPA canonical pathways, with the exception of 9 pathways that did not overlap between normal and obese diets. Pathways with the highest *z* scores (predicted to be activated) for the normal but not for obese diet include SUMOylation of immune response proteins, TAK1-dependent IKK, NF-kappa-B activation, and toll-like receptor signaling. In contrast, pathways predicted to be activated for obese but not for normal diet include HiF1a, ceramide, and erythropoietin signaling.

Comment

Principal findings

Using a mouse model of intrauterine inflammation and preterm birth, we demonstrated that maternal obesity magnifies perinatal neuroinflammation. This was independent of paternal contribution or variables, such as gestational age, that were experimentally controlled. Our findings suggest that maternal obesity is a risk factor for fetal brain injury in the setting of intrauterine infection.

Clinical implications

Obesity is a major public health issue that affects approximately 40% of adults in the United States.[30] Obesity is associated with poor pregnancy outcomes, including intra-amniotic infection, premature birth, cesarean delivery, and maternal surgical site infections.[15,16,31] In offspring, maternal obesity is associated with neurologic issues, such as

blue if down-regulated. For the group with obesity, genes up-regulated with a fold change of >1and a *P*<.05 are annotated in *red*, whereas all genes down-regulated with *P*<.05 are in *blue*. The *dotted horizontal lines* denote a *P*=.05, and the *dotted vertical lines* reflect a fold change of 1.

LPS, lipopolysaccharide; *PBS*, phosphate-buffered solution; *PCR*, polymerase chain reaction.

Leonard. Increased fetal neuroinflammation with maternal obesity in a murine model of preterm labor. Am J Obstet Gynecol Glob Rep 2024.

FIGURE 4
Neuroinflammatory expression pathway analysis

Bubble charts reflect the ingenuity pathway analysis (IPA) canonical pathway analysis for the NanoString DEG results from normal and obese diets between LPS and PBS exposure. Pathways are sorted vertically by *z* score (activity). *Darker color gradients* reflect a greater association for each activity based on the distance of the *z* score from 0, whereas *white bubbles* lack a *z* score. *Bubbles* are color coded to denote *z* score patterns among active (*orange*), no activity (*gray*), and inactive (*blue*) pathways. Moreover, pathways are sorted horizontally by the *P* value shown in −log. Represented are the top pathways with a −log(*P* value) of >7. The size of each *bubble* varies by the number of DEGs associated with each pathway and reflect only those with an adjusted *P*<.05 from the NanoString analysis. *Arrowheads* point to pathways that do not overlap between normal and obese diets.

Leonard. Increased fetal neuroinflammation with maternal obesity in a murine model of preterm labor. Am J Obstet Gynecol Glob Rep 2024.

cognitive deficiency and behavioral disorders.[32−34] However, the effect of maternal obesity on neurodevelopment alone or in conjunction with comorbidities, such as premature birth, remains unclear.

With premature birth, there is a substantial risk of neonatal morbidity and mortality.[35] Preterm birth is often mediated by an underlying bacterial infection and related intrauterine inflammation.[35] Hillier et al[36] found a compelling association between the onset of labor before 34 weeks of gestation and intrauterine inflammation. Here, the overwhelming majority of preterm deliveries showed elevated levels of IL-1B, IL-6, and TNF concurrently with bacterial infection.[36] These inflammatory cytokines potentiate destructive immune responses, particularly in the developing brain, when evaluated in animal models.[19,35,37−39] Neuroinflammatory injury is believed to be the cause of central nervous system−related adverse fetal outcomes, such as cerebral palsy.[40]

Independently, prematurity and maternal obesity can negatively affect brain development.[41−43] However, it is unknown whether obesity increases the risk of fetal neuroinflammatory injury in cases of preterm birth. In fetal brains from dams with obesity, we observed a significant up-regulation of inflammatory genes and processes, notably *Il6*.[21] Previous studies have indicated that elevated levels of IL-6 initiate a complex cascade of pathways that result in epigenetic alterations, including downstream methylation of key promoters related to neuronal gene expression.[38,44] Such mechanisms may explain the link between maternal immune activation of IL-6 and the modulation of fetal brain development.[38] Therefore, in the context of preterm birth, increased levels of IL-6 with maternal obesity may impair neurodevelopment.

Although factors that govern the severity of fetal neuroinflammatory injury remain to be fully characterized, our data suggest that maternal health is key. Pregnancy itself is an immunologic phenomenon that requires a delicate balance between pro- and anti-inflammatory pathways. Both preterm labor and maternal obesity are associated with inflammatory biases that affect the intrauterine environment.[31,36] It is possible that adverse fetal neurologic sequelae related to the maternal environment are potentiated by the maternal-fetal interface. This process may damage the intrauterine environment and influence epigenetic modifications that lead to unfavorable pregnancy outcomes.[45]

Research implications

In the absence of LPS, we did not observe differences, suggesting that these potent inflammatory factors are not altered by maternal obesity alone within the fetal brain. As demonstrated by previous adaptations of this model, LPS-mediated intrauterine inflammation significantly up-regulated *Il1b*, *Il6*, and *Tnf* in fetal brains (Figure 2, A).[23,27] Among these key cytokines, only *Il6* was different with obesity and LPS.[23] These changes occurred without increases in fetal weight, suggesting that maternal obesity can influence the developing brain in the absence of fetal macrosomia. Maternal obesity alone elevates *Il6* expression in mouse placentas, which may increase systemic responses in response to intrauterine inflammation.[46] Despite the relationship between LPS and Tlr4 signaling, differences within fetal brains attributed to maternal obesity do not coincide with *Tlr4* expression.[25]

To gain broader insight into the effect of maternal obesity on the developing brain, we analyzed 2 gene expression panels reflecting metabolism and immunity. There were few metabolic differences with LPS, irrespective of maternal status, whereas immune-related genes were highly up-regulated with obesity. This comparison suggests that fetal neuroimmune and metabolic responses to intrauterine inflammation are not mutually exclusive or equally affected by maternal obesity. Based on these observations, we expanded our analysis of neuroimmune-related genes rather than metabolic. With the expanded analysis, cross-platform consistencies and inconsistencies were noted. The immune panel analysis (Figure 2, B) was underpowered compared with the NanoString analysis (Figure 3). This may relate to differences for certain genes, such as *Ccl3*, which was significant only for the obese diet using the immune panel, but for both diets using the NanoString (Supplemental methods and data file). The exception is *Tnf*, which was significantly different by qRT-PCR (Figure 2, A) but was not detected by the NanoString (Figure 3), which may reflect cross-platform differences and sensitivities. Overall, the gene expression results support a relationship between maternal health and fetal neuroinflammation (Figure 3). Certain genes (Casp4, Spp1 and Lcn2) up-regulated only with obesity and LPS relate to white matter injury and may increase the risk of this pathology common among children born prematurely.[47] Specifically, Casp4 (aka Casp11) can initiate oligodendrocyte death, whereas Spp1 mediates microglial dysfunction and is linked to poor myelination in humans.[48−50] Concurrently, Lcn2 knockout animals have shown resistance to white matter damage compared with wild-type mice with subarachnoid hemorrhage injury.[51] In humans, elevated levels of LCN2 in the cerebral spinal fluid correlate with age-related white matter decline,[52] and LCN2 is also observed in the circulation of neonates born prematurely.[53]

The predicted IPA canonical pathways and their activities (delineated by the *z* score) were similar between normal and obese diets. The activation of several overlapping cytokines and inflammatory signaling pathways was biased by our use of a targeted panel of genes that were mostly up-regulated with LPS exposure. Despite this and similarities in activity and statistical significance (*P* value) for most pathways, there are notable differences. Among the most significant pathways depicted in Figure 4, the activation of HiF1a signaling was predicted with the obese but not for the normal diet. In contrast, inactivation of peroxisome proliferator-activated receptor (PPAR) signaling is predicted for the normal but not for the obese diet. Studies indicate that HiF1a promotes brain injury,[54] whereas PPAR

signaling can be neuroprotective.[55] This pattern suggests that the influence of maternal diet on perinatal neuroinflammation may be selective and even opposing. Further research is needed to determine the role of select pathways, including HiF1a and PPAR, within the context of maternal health and perinatal brain injury.

Strengths and limitations

A strength of our study is the comparison of high-fat diet–induced obesity, which is the leading cause of excessive weight gain in humans.[56] Using a well-validated experimental mouse model, the differences can be attributed to maternal obesity. In contrast, human studies may not be able to control for variables that also affect neurodevelopment, such as gestational age at delivery and genetic disposition.[17,57] Furthermore, we eliminated paternal influence as a contributing factor using the same sires without obesity for both normal and obese dams. The controls provide additional insight regarding maternal influence on fetal neuroimmune responses and lack thereof with obesity alone. This was examined using several gene expression approaches, including the NanoString digital analysis platform.[58]

The major limitation of our study is that observations in animal models do not always translate to human biology and that LPS-mediated inflammation may not reflect the complexities of intraamniotic infection. In addition, we only compared a high-fat diet along with intrauterine inflammation using LPS from *E coli*. It is possible that our findings do not extend to obesity linked to genetic or other dietary types or to other etiologies of perinatal neuroinflammatory injury.[18] Our analysis of immune and metabolic genes was not comprehensive and does not account for all possible developmental changes related to maternal obesity. Correspondingly, our reliance on targeted gene expression approaches may relate to the lack of overwhelming DEGs and pathways observed in obesity. Finally, our analysis relies on gene expression, which may not reflect protein translation and necessitates further verification.

Conclusions

Our findings suggest that maternal obesity compounds fetal neuroinflammatory responses to cytotoxic stimuli in utero. The combination of obesity and intrauterine inflammation remains ill understood, yet it may compound fetal injury and lifelong comorbidities associated with premature birth. Further examination of maternal obesity as a potentially modifiable risk factor to mitigate the severity of neurologic-related fetal outcomes with prematurity is warranted. ■

CRediT authorship contribution statement

Katherine M. Leonard: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Stacey S. Schmiedecke:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Rebecca L. Talley:** Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Jennifer R. Damicis:** Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Robert B. Walton:** Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Irina Burd:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing. **Peter G. Napolitano:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. **Nicholas Ieronimakis:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.xagr.2024.100361.

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