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Full-length Article

Maternal high fat diet exposure is associated with increased hepcidin levels, decreased myelination, and neurobehavioral changes in male offspring

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ABSTRACT

Maternal obesity induces chronic inflammatory responses that impact the fetus/neonate during the perinatal period. Inflammation, iron regulation, and myelination are closely interconnected and disruptions in these processes may have deleterious effects on neurodevelopment. Hepcidin levels are increased in response to inflammation causing subsequent decreases in ferroportin and available iron needed for myelination. Our current studies were designed to test the hypotheses that: 1) maternal high fat diet (HFD) prior to and during pregnancy is sufficient to induce inflammation and alter iron regulation in the brain of the offspring, and 2) HFD exposure is associated with altered myelination and neurobehavioral deficits in the offspring. Our data revealed modest increases in inflammatory cytokines in the serum of dams fed HFD prior to pregnancy compared to dams fed a control diet (CD). Early increases in IL-5 and decreases in IL-10 were observed in serum at PN7 while IL-5 remained elevated at PN21 in the HFD-exposed pups. At PN0, most cytokine levels in whole brain homogenates were higher in the pups born to HFD-fed dams but were not different or were lower than in pups born to CD-fed dams at PN21. Conversely, the inflammation mediated transcription factor Nurr77 remained elevated at PN21. At birth, brain hepcidin, ferroportin, and L-ferritin levels were elevated in pups born to HFD-fed dams compared to pups born to CD-fed dams. Hepcidin levels remained elevated at PN7 and PN21 while ferroportin and L-ferritin levels were lower at PN7 and were not different at PN21. Decreases in myelination in the medial cortex were observed in male but not in female pups born to maternal HFD-fed dams at PN21. These structural changes correlated with changes in behavior (novel object recognition) in at 4 months in males only. Our data indicate that maternal obesity (HFD) results in disruption of iron regulation in the brains of the offspring with structural and neurobehavioral deficits in males.

1. Introduction

Pre-pregnancy maternal obesity is a worldwide problem affecting approximately one third of women of childbearing age in the United States (Ogden et al., 2013). Maternal obesity confers significant maternal and fetal/neonatal health risks during the perinatal period including preeclampsia/gestational hypertension, gestational diabetes mellitus (Scott-Pillai et al., 2013), preterm delivery (Cnattingius et al., 2013; Khatibi et al., 2012), stillbirth (Salihu, 2011; Yao et al., 2014), and delivery complications such as need for cesarean section or shoulder dystocia (Magann et al., 2013; Ruager-Martin et al., 2010; Scott-Pillai et al., 2013; Stothard et al., 2009; Turcksin et al., 2014). Infants born to obese mothers are also at an increased risk for long-term health problems including obesity and metabolic syndrome (Boney et al., 2005). More recent research indicates that children born to obese mothers are also at an increased risk for impaired neurodevelopment and

Abbreviations: HFD, high fat diet; CD, control purified diet; DIO, diet induced obesity; PFA, paraformaldehyde; PBS, phosphate buffered saline; SEM, standard error of the mean.

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learning disabilities (Hinkle et al., 2013; Reynolds et al., 2014), although the biological mechanisms involved are not well understood.

Obesity is a state of chronic inflammation and offspring of obese mothers are exposed to inflammatory mediators throughout gestation and lactation. Animal studies suggest that the inflammation induced in the offspring in response to maternal obesity is due to either high fat diet or the obesity itself. In rodents, increases in specific cytokines such as IL-1b or IL-6 in the brain are correlated to impaired cognition and memory suggesting that obesity-related inflammation directly influences neurological function (Bilbo and Tsang, 2010; Pistell et al., 2010; White et al., 2009; Yan et al., 2011). Diet-induced obesity (DIO) rodent models have thus far provided insights into the potential mechanisms underlying these associations including decreased DNA methylation in the brain (Carlin et al., 2013), alterations in neural networks that regulate food intake (Stachowiak et al., 2013), and changes in hippocampal development in the offspring (Niculescu and Lupu, 2009) but these pathways have not been extensively defined.

Inflammation, iron regulation, and myelination are closely interconnected and disruption in these processes may have deleterious effects on neurodevelopment (Lieblein-Boff et al., 2013; Urrutia et al., 2013; Wang et al., 2008). Importantly, hepcidin is a critical hormone that regulates iron homeostasis by negatively controlling ferroportin expression (Oates, 2007). Hepcidin levels typically decrease during normal pregnancy, likely due to a pregnancy-related iron deficiency (van Santen et al., 2013). Hepcidin signaling is increased in the setting of inflammation (Nemeth et al., 2004; Urrutia et al., 2013; Wang et al., 2008) and leptin levels (Chung et al., 2007) are also increased in obese pregnant women (Dao et al., 2013). Since hepcidin is primarily stored in oligodendrocytes and iron is an essential component for myelination, changes in hepcidin levels may negatively impact neurodevelopment by reducing myelination (Lieblein-Boff et al., 2013; Oates, 2007; Rossi, 2005). Urrutia et al. reported a change in iron content and a disruption in iron regulation in response to acute inflammation which resulted in iron accumulation in neurons and microglia (Urrutia et al., 2013). They also reported that disruption of iron homeostasis was due to changes in hepcidin and ferroportin expression. Wang et al. identified increases in hepcidin in the cortex and substantia nigra due to LPS administration (Wang et al., 2008). Leiblein-Boff et al. established relationships between inflammation, iron homeostasis and neurological behaviors in a development model induced by bacterial infection (Lieblein-Boff et al., 2013). These studies found that inflammation decreased iron availability, increased locomotor activity, decreased motor coordination and resulted in hypomyelination and decreased oligodendrocyte number in the white matter and motor cortex of the mice in adulthood. Collectively, these data strongly support a mechanistic link between obesity, inflammation, iron homeostasis, hypomyelination, and neurological function. Thus, the present studies tested the hypothesis that maternal obesity associated inflammation would result in inflammation in the offspring which would, in turn, be associated with altered iron availability, myelination, and neurocognitive behavior.

2. Materials and methods

2.1. Animals

All animal studies were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee at The Research Institute at Nationwide Children's Hospital (Columbus, OH). Three-week-old female C57Bl/6J mice were purchased from Jackson Labs. Mice were housed in the facility for one week to allow habituation. At four weeks of age, mice were randomly assigned to either the high fat (Harlan Teklad TD.06414, 60% calories from fat, Madison, WI) or control diet (Harlan Teklad TD.08806, 10% calories from fat, Madison, WI). The mice were weighed weekly. Diets were stored at 4 °C and diet in cages was replaced weekly to prevent degradation. At the start of the ninth week on experimental diet, the female mice were paired for breeding continuously for two weeks. A subset of female mice was sacrificed prior to breeding for blood and tissue collection prior to pregnancy. Male mice were maintained on standard chow except for the two week breeding periods when they were exposed to the respective experimental diet. Visibly pregnant mice were individually housed prior to giving birth. Pups were weighed and euthanized at postnatal days (PN) 0, 7, and 21. A subset of male and female pups were weaned onto standard chow (Teklad 2020X) at day 21 and underwent behavioral testing at 12 weeks of age then returned to the home cage and were euthanized at 4 months (16 weeks) of age for tissue collection.

Mice were anesthetized with intraperitoneal injections of ketamine/xylazine (150 mg/kg: 15 mg/kg, respectively) for tissue collection. Cardiac puncture was performed after anesthesia for blood collection. Brains were either collected and frozen in liquid nitrogen immediately or prepared for fixation. For brains collected on PN7, the intact skulls were submerged in 4% paraformaldehyde (PFA) for 24 h after which the skulls were removed and the brains were returned to 4% PFA for an additional 24 h of fixation. Brains were washed and stored in phosphate buffered saline (PBS) and embedded in paraffin. For brains collected at PN21 or later, whole body perfusions were performed with cold saline followed by 4% PFA. Brains were quickly removed and placed in 4% PFA for 24 h prior to washing with PBS and embedding in paraffin. Coronal sections were cut serially at 4 μm from paraffin blocks and collected on positively charged slides.

2.2. Real time quantitative polymerase chain reaction

RNA was isolated from brain tissues using TRIzol (Invitrogen) and RNeasy kit (Qiagen) following the manufacturer's protocols. RNA (2 μg) was reverse transcribed per instructions using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). PCR with Maxima SYBR green master mix (ThermoScientific) was performed on an Eppendorf Realplex Master Cycler using custom DNA primers (Integrated DNA Technologies). Primer sequences are provided in Table 1.

2.3. ELISA

Dams were fasted for four hours prior to serum collection for leptin/insulin testing. After lethal anesthesia, blood was collected through cardiac puncture. Serum was separated and analyzed using the Meso Scale Discovery mouse metabolic kit (K15124C-1) and the proinflammatory panel kit V-PLEX™ (K15048D-1).

2.4. Western Blot

Western blots analyses were performed as previously described (Graf et al., 2014). Whole brain homogenates were separated by electrophoresis on 4–12% bis-tris gels and transferred to nitrocellulose membranes. Membranes were probed with antibodies to proteolipid protein (1:500, Abcam, ab28486). Membranes were then probed with a HRP-conjugated goat anti-rabbit secondary (1:12,000; Biorad, 170-6515) and developed using enhanced chemiluminescence (GE Healthcare). Relative densitometry was determined using Image Quant Software (Molecular Dynamics).