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High fat diet-induced maternal obesity alters fetal hippocampal development

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\textbf{A B S T R A C T}

The importance of maternal nutrition for fetal brain development is increasingly recognized. Previous studies have suggested that maternal obesity or maternal exposure to obesogenic diets may permanently alter brain structure and function in the offspring. To test whether maternal exposure to a high-fat diet, prior and during gestation, alters fetal hippocampal development, we fed 8-week old C57BL/6 females with a high-fat diet (60% calories from fat) for 10 weeks prior to mating and 17 days after. Fetal brains at embryonic day E17 were used to determine developmental changes in the hippocampus. We report that maternal exposure to the high-fat diet induced small for gestational age (SGA) status and fetal resorption. The proliferation of neural progenitors was increased in the neuroepithelium from hippocampus and cortex in fetuses from mothers fed the high-fat diet when compared to controls, but decreased within the dentate gyrus (DG). Apoptosis in the hippocampus was decreased (Ammon’s Horn and fimbria). The differentiation of calretinin-positive neurons within the DG was also decreased.

These data indicate that, under the influence of a maternal high-fat diet administered prior and during gestation, fetal hippocampal development is altered at embryonic day 17, as indicated by region-specific changes in proliferation of neural precursors, decreased apoptosis, and by decreased neuronal differentiation within the dentate gyrus.

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\section{1. Introduction}

Maternal nutritional status during gestation is an essential player that regulates fetal brain formation, with long-lasting consequences upon memory, learning, and brain senescence (Gordon, 1997; Guesry, 1998; Mattson, 2003; Mattson and Shea, 2003).

Few epidemiological studies suggested that a fat-rich diet (total and saturated fat) might accelerate age-related cognitive decline and onset of dementia in humans (Kalminijn, 2000; Solfrizzi et al., 2003). In several prospective or nested epidemiological studies, a high BMI was linked to increased risk of dementia when measured at least 10 years prior to a clinical diagnosis (Gustafson, 2008)). Less data is available in humans regarding the role of obesity and high fat diets in brain development. Obesity in children lowered plasma brain-derived neurotrophic factor (Bdnf) levels (El-Guesry, 1998; Mattson, 2003; Mattson and Shea, 2003).

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In adult rats (males only), a high-fat diet altered neurogenesis in the hippocampus by decreasing the proliferation of NeuN+ neuronal-specific committed progenitors (Lindqvist et al., 2006). When on a high-fat, dextrose rich diet, adult male rats and mice decreased Bdnf levels in the cortex and hippocampus (Yu et al., 2009), associated with alterations in discrimination reversal (Kanoski et al., 2007) and with reduced hippocampal spatial learning performance (Molteni et al., 2002). Conversely, moderate caloric restriction was reported to increase Bdnf levels and neurogenesis in the adult hippocampus (Lee et al., 2000). Similarly, adult mice on a high-fat diet failed to perform an operant barpressing task (Mielke et al., 2006). In these models, obesity, high triglycerides, insulin resistance, and hyper-leptinemia were present (Matyskova et al., 2007; Yamato et al., 2007), rendering them as a suitable approximation for the metabolic syndrome in humans.

A very limited number of studies indicated that maternal obesity might be another important trigger that altered brain development in the offspring. When pregnant rats were fed a high-fat diet beginning with gestational day 5, their fetuses had increased neural progenitor proliferation (NeuN positive cells) within the hypothalamus beginning with embryonic day 14 (E14), and this outcome was still present at later stages (birth day, P0) in selected hypothalamic areas (paraventricular nucleus and perifornical lateral hypothalamus)(Chang et al., 2008). In the same study pups born and cross-fostered to lactating dams on a control diet exhibited less NeuN-specific neurogenesis in the hypothalamus than pups from...
high-fat mothers maintained on a high-fat diet during lactation (Chang et al., 2008). Pups from C57BL/6 females exposed to a high-fat diet prior and during gestation, and during lactation, exhibited long-lasting (until postnatal day P70) decreased neurogenesis (proliferation of neural precursors) in the dentate gyrus (Tozuka et al., 2009). The exposure of suckling pups to a maternal high-fat diet may also play a role in neonatal brain development (Walker et al., 2008). These studies indicated that maternal exposure to a high-fat diet not only altered fetal neurogenesis, but the exposure during the lactating period also played an important role for postnatal brain development.

In this study, we sought to determine whether the administration of a high-fat diet, prior and during gestation, induces alterations in the neurogenesis of fetal hippocampus at gestational day 17 (E17). Based on published data, we have hypothesized that maternal obesity induces developmental delay in the fetal hippocampus, as determined by using markers of cell proliferation, apoptosis, and early neuronal differentiation.

2. Experimental procedures

All reagents were purchased from Sigma–Aldrich (St. Louis, MO) if not otherwise specified.

2.1. Animals

C57BL/6 females (8 week old, Charles River, Wilmington, MA) were randomly assigned into two feeding groups: a control group (CT) receiving a low-fat diet (10% calories from fat, D12450B, Research Diets, New Brunswick, NJ), and a high-fat diet group (HF) receiving a high-fat diet (60% calories from fat, D12492, Research Diets). The composition of both diets is indicated in Table 1, as provided by the manufacturer. Mice were maintained in a climate-controlled environment and exposed to a 12 h light/dark cycle daily. All animal protocols were approved by the UNC Institutional Animal Use Committee. Mice were housed four per cage and had access to food and water ad libitum for the entire exposure period. Both groups received the specified diets for a 10-week period. During the last 2 weeks of the dietary treatment, estrus cycle was induced and synchronized among the females from same cage by using dirty male bedding containing male urine (Whitten effect). After 10 weeks the females were bred overnight (a 12 h period) with males that were maintained at all times on the CT diet. The following morning, the males were separated from females. Pregnant females were identified by either the presence of the vaginal plug (gestation day E0) or by palpation beginning with gestation day E11. All females were maintained during gestation on the same diet received before.

Body weight was monitored every week prior and during gestation. On day E17 pregnant mice were anesthetized with a single intraperitoneal injection of ketamine (100 mg/kg, body weight) and xylazine (10 mg/kg body weight) (Henyink Schein Inc., Mahwah, NY). The mice were kept on a heating pad to maintain body temperature. The uterine horns were exposed by a midline abdominal incision and the fetuses were removed individually. After recording fetal body weight, fetuses were decapitated and half of the fetal skulls from each litter were frozen and immersed in 4% formaldehyde for 12 h. The second day fixed fetal brains were removed from the skulls, and stored for further paraffin embedding in 0.1 mol/L phosphate buffer, pH 7.4. Fetal bodies were used for sex identification.

2.2. Immunohistochemistry

Fixed male fetal brains were paraffin-embedded and 5 μm coronal serial sections were cut and applied on glass slides for immunohistochemical assays. Because the development of the hippocampus, and especially of the dentate gyrus, has a three-dimensional gradient (temporal to septal, suprapyramidal to infrapyramidal, and along the radial dimension for dentate gyrus) (Stanfield and Cowan, 1979), and in order to prevent the detection of false changes between groups due to possible differences in the localization of similar anatomical areas along the anterior to posterior axis, we used hippocampal sections selected from the middle location, ensuring the highest possible correspondence between the two groups. Alternating sections were used for labeling with the markers described below, such to ensure relevance to the same anatomical localization for all markers.

Mitosis in the ventricular/subventricular (VZ/SVZ) and dentate gyrus (DG) areas within hippocampus, and within the cortical VZ area, was determined using a rabbit antibody against phosphorylated histone H3 (Ser 10, pH 3, Millipore, Temecula, CA), Ser-10 is phosphorylated first in the G2/M phase of the cell cycle then progresses along the chromosomal arms until it spreads to the whole chromosome (Hendzel et al., 1997). A rabbit antibody against activated Caspase-3 (Cell Signaling Technology, Danvers, MA) was used for assessment of apoptosis. The early differentiation of neurons within the dentate gyrus (DG) was measured using a goat primary antibody against mouse calretinin (N-18, Santa Cruz Biotechnology, Santa Cruz, CA). Secondary fluorescent antibodies were used as it follows: goat anti-rabbit Cy3 conjugated (AP132C, Millipore) for pH 3 and Caspase-3 labeling, and Alexa Fluor 594 chicken anti-goat (Invitrogen, Carlsbad, CA) for detection of calretinin labeling. The following common protocol was used for all immunohistochemical determinations: slides were deparaffinized in xylene twice for 5 min each, followed by incubation for 5 min in 10% sucrose, 10% DMSO for all immunohistochemical procedures.

2.3. Image and statistical analysis

Image analysis of fetal brain sections was performed using a Zeiss Axios Imager A1 microscope (Carl Zeiss, Thornwood, NY) with Plan-Neofluar 20×/0.75 NA objective and 40×/1.3 NA objectives. Images were collected and analyzed as it follows: for the assessment of pH 3 within the DG and hippocampal and cortical VZ/SVZ areas, positive cells were counted in both hemispheres on three alternating sections and the values were averaged to obtain a single value/hippocampus (region)/animal. Activated Caspase-3 positive cells were counted similarly within the following hippocampal regions: Fimbria (FF), Ammon's Horn (CA), intermediate zone (IZ), DG, and V2/SVZ areas. For calretinin assessment, since its abundance within the DG did not clearly allow the counting of positive cells, we used the integrated optical density (IOD) as proxy marker for protein levels, as previously described (Niculescu et al., 2006), using the ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis was performed using the Student’s t-test, after testing for equality of variances (JMP 7.0.1, SAS Institute, Cary, NC). The significance of fetal resorptions was determined using the Fisher exact test (EpiInfo, CDC, Atlanta, GA).

3. Results

3.1. Maternal weight and gestation outcomes

A total of 24 C57BL/6 mice (8-week old) were randomly assigned to either the control group receiving the low-fat diet (CT, n = 12), or to the group receiving the high-fat diet (HF, n = 12). Body weight was measured at the beginning of the treatment (week 0), and at the end of each week, for a total of 10 weeks. Within the HF group, a mouse was sacrificed during the 10-week period due to stunting followed by loss of weight. Therefore, only 11 mice in the HF group were included in the analysis of weight. During the 10-week exposure to the high-fat diet, the weight gain in the HF group was higher than in the CT group (Fig. 1), reaching an average of

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Table 1

| Composition of diets. The list includes only the content in protein, carbohydrate and fat, and ingredients with different concentrations between the two diets. Complete formulations can be found at http://researchdiets.com/. For the list of ingredients, numbers with decimals were rounded to the nearest integer. |
|----------------|----------------|----------------|
| **Formulas**  | **Low-fat diet** | **High-fat diet** |
|               | (CT, control)   | (HF, high fat)  |
|               | g% kcal        | g% kcal        |
| Protein       | 19.2           | 20             |
| Carbohydrate  | 67.3           | 70             |
| Fat           | 4.3            | 10             |
| Total         | 3.85           | 5.24           |
| **Ingredient**| **g**          | **g**          | **kcal** |
| Corn starch   | 315            | 1260           | 0        |
| Maltodextrin 10 | 35              | 140            | 125      |
| Sucrose       | 350            | 1400           | 69       |
| Soybean oil   | 25             | 225            | 25       |
| Lard          | 25             | 180            | 245      |
|               |                 |                 | 2205     |
30.9 g ± 1.6 SE, compared to an average body weight in the CT group of 21.6 g ± 0.4 SE.

At the end of the 10-week exposure period, all females were bred with males that received the CT diet. Out of the 23 females, 13 became pregnant (7 on CT diet and 6 on HF diet). While no resorbed fetuses were identified in the CT group, the HF groups had an average of 0.83 resorbed fetuses per pregnant mouse (represented by five resorptions distributed across three pregnancies, from a total of 49 fetuses). When the weight of viable fetuses was compared between groups, the HF group had a lower fetal body weight than controls (Fig. 2C).

Although the weight of fetal brains (including the cerebellum) was not significantly changed in the HF group (50.9 mg ± 4.5 SE in the HF group versus 58.2 mg ± 4.8 SE in the CT group, all non-fixed fetal brains included), a similar trend with the changes in fetal body weight was observed.

### 3.2. Cellular proliferation

In order to establish whether maternal high-fat diet or the obese phenotype has an impact upon the development of fetal hippocampus and cortex, we measured cell proliferation within the dentate gyrus (DG) and the ventricular/subventricular (VZ/SVZ) areas of hippocampus and cortex. At E17, hippocampal neurogenesis is localized mainly within the DG and VZ/SVZ areas (Rodier, 1977). We therefore measured cell proliferation within these areas, and also included the VZ/SVZ cortical areas, using a G2/M phase-specific mitotic marker (pH 3). Using only male fetuses (each from a different litter, n = 7 and n = 6 for the CT and HF groups, respectively), pH 3-positive cells were counted and averaged within both groups (Fig. 3). In the VZ/SVZ hippocampal areas, the HF group had more mitotic cells than the CT group (17.81 ± 1.94 versus 11.88 ± 1.67, respectively). Identical results were obtained for mitosis within the cortical VZ/SVZ area (17.86 ± 2.00 versus 11.75 ± 1.73, respectively). Conversely, mitosis in the dentate gyrus (DG) was decreased in the HF group when compared to CT group (2.97 ± 0.63 versus 5.03 ± 0.35).

### 3.3. Apoptosis

Apoptosis within the fetal hippocampus was investigated using an antibody against activated Caspase-3. Positive cells were counted separately for each of the following hippocampal areas: Fimbria (Fi), Ammon’s Horn (CA), intermediate zone (IZ), DG, and ventricular/subventricular (VZ/SVZ) areas. Only male fetal brains were used, with each brain from a different litter (n = 7 and n = 6 for the CT and HF groups, respectively). Statistical significance (**p < 0.01**) was tested by Student’s t-test, after testing for equal variance. CT, control diet; HF, high-fat diet; error bars, SE.
areas). Apoptosis in the hippocampus of the HF fetal brains was decreased compared to the CT group (Fig. 4). Specifically, the number of apoptotic cells was decreased in the Ammon's horn (CA) and Fimbria (Fi) of the HF fetal hippocampi. Within the CA area, apoptosis was decreased 80% (0.35 ± 0.15 in HF group versus 1.74 ± 0.42 in CT group, respectively), while in the Fi area the number of apoptotic cells decreased 81% when compared to control (0.13 ± 0.07 in HF groups versus 0.67 ± 0.25 in CT group). The total cell proliferation within the hippocampus and the adjacent cortical ventricular area was assessed using fluorescent immuno-labeling against phosphorylated histone H3 (pH 3, specific marker for G2/M-phase). Three sections from anatomically identical hippocampal regions were used from each fetal brain (n = 7 CT group, n = 6 HF group, all males, E17, right and left hemispheres each). The total number of positive pH 3 cells was counted within the dentate gyrus (DG), the ventricular/subventricular hippocampal area (VZ/SVZ-hippo), and the cortical ventricular and subventricular areas adjacent to hippocampus (VZ/SVZ-cortex). Cell proliferation decreased in the DG of fetuses from obese mothers (HF group), while cell proliferation of the VZ/SVZ areas increased in both hippocampus and cortex. The picture represents three merged images (using the red, green, and blue filters) of the same hippocampal section (CT group), indicating the specificity of pH 3 staining (red cells), while red blood cells are auto-fluorescent in both spectra (red and green). Blue fluorescence indicates nuclear staining with diammino-phenylindole DAPI). *p < 0.05, **p < 0.01, respectively, by Student's t-test. Error bars indicate standard errors (for interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Using an antibody against mouse calretinin, followed by secondary binding to a fluorescent antibody, calretinin levels within the dentate gyrus (DG) were measured in E17 fetal brains. Since calretinin positive neurons are abundantly present in DG, optical density was used to determine calretinin levels, rather than counting the number of positive cells (see experimental procedures). All images were collected under the same exposure microscope settings. ImageJ software was used to determine integrated optical density (IOD) within similar DG areas across sections, where the auto-fluorescence of red blood cells was used to normalize between images. The image depicts specificity of calretinin staining (CT group). The red blood cells auto-fluorescence was differentiated from calretinin signals using merged images (red and green filter), where these are indicated by yellow (combined green and red channels). Specificity of calretinin staining (red) is demonstrated by anatomical localization and by the lack of secondary fluorescence on the green channel. Significance was assessed by Student's t-test for *p < 0.05. The IOD values represent mean pixel density per selection area (arbitrary units). Error bars denote standard error (for interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).
number of apoptotic cells within the hippocampus was also decreased in the HF group when compared to CT group (1.43 ± 0.42 and 3.63 ± 0.86).

3.4. Calretinin assessment

Calretinin immuno-staining was used in order to determine whether early neuronal maturation is altered in the dentate gyrus of E17 fetuses from females exposed to the high-fat diet. At E17–E18, calretinin-expressing neurons are mostly confined within the outer marginal zone (omz) and dentate marginal zone (dmz) of the DG (Soriano et al., 1994). Only male fetal brains were used (n = 7 in CT group and n = 6 in HF group). Because of the abundance of calretinin-expressing neurons and their overlapping, calretinin levels were measured using integrated optical density (IOD), as previously published (Niculescu et al., 2006). Calretinin levels in the DG of the HF were 75% of those in CT group: 3.81 ± 0.34 IOD arbitrary units in HF group versus 5.08 ± 0.38 IOD units in the CT group, respectively (Fig. 5).

4. Discussion

The exposure of C57BL/6 females to a high-fat diet containing 60% calories from fat induced maternal obesity, and fetal resorption and SGA status in fetuses at gestational day 17 (E17). Although litter size was identical in both feeding groups, more than 10% of fetuses were resorbed in the HF group at the time of sacrifice (day E17, Fig. 2A). When only viable fetuses were accounted, maternal exposure to a high-fat diet also induced intra-uterine growth restriction, as determined by fetal body weight (Fig. 2C).

According to published data, the most plausible mechanism responsible for fetal resorption is related to the induction of a pro-inflammatory environment by maternal diabetes, during embryo implantation (Jawerbaum and Gonzalez, 2006). Another aspect to be considered is the potential alteration in energy expenditure, related to gestational outcomes. In C57BL/6J mice a higher basal metabolic rate (BMR) during gestation was associated with gestational weight loss indicative of fetal resorption (Johnston et al., 2007). The increased BMR in mice receiving high-fat diets is probably due to induced uncoupling protein-1-mediated thermogenesis (Kus et al., 2008).

In the present study, hippocampal development was assessed only in fetal males. Although no data are available to suggest that a maternal high-fat diet alters fetal brain development in a sex-specific manner, studies involving intrauterine exposure to maternal hyperglycemia (expected in our study) indicated sex-dependent deficits in learning and memory, suggesting that the neurodevelopment in male and female progeny may be differently affected (Kinney et al., 2003).

The assessment of hippocampal development using markers of cell proliferation (phosphorylated histone H3, pH 3), apoptosis (activated Caspase-3), and early neuronal differentiation (calretinin) revealed marked differences in hippocampal formation between male fetuses from HF and CT mothers. These outcomes followed a total exposure to the HF diet of approximately 12.5 weeks (10 weeks prior to pregnancy and until embryonic day 17). The assessment of cell proliferation revealed interesting, divergent outcomes in the V2/SVZ (increased neurogenesis) versus DG area (decreased proliferation) in the HF group.

Several studies using rodent models have addressed the relationship between high-fat dietary intakes or obesity, and hippocampal development. However, most of them did not refer to prenatal, but rather to postnatal development, indicating that high-fat diets negatively alter the neurogenesis within the dentate gyrus of adult rodents, and these changes are persistent (Lindqvist et al., 2006; Yu et al., 2009; Lee et al., 2000).

Only few studies have addressed the hypothesis that high-fat maternal intakes, prior and during gestation, may alter fetal brain development. The short exposure of pregnant Sprague Dawley rats to a high-fat diet (50% fat with mainly lard, beginning embryonic day E6) increased the proliferation of cells within the hypothalamic areas of E11–E15 rat fetal brains, which is the period of peak cell birth in rat hypothalamus (Chang et al., 2008). In a second study, which is the closest to our model, Tozuka et al reported that, when 4-week old C57BL/6 mice were fed with a similar high-fat diet (57.5% calories from fat) for 6 weeks prior, and during gestation, the neurogenesis in the DG was decreased in the offspring, beginning with postnatal day P21 and until P70, as determined by BrdU incorporation (Tozuka et al., 2009). However, in opposition to our findings, Tozuka et al. reported no differences in DG neurogenesis at embryonic day E18 (one day later than our time point at E17), and no changes in fetal body weight at E18 (Tozuka et al., 2009). Several factors could account for differences in our reported outcomes. The most significant difference is the duration of dietary exposure prior to gestation (4 weeks longer exposure in our study, resulting in a bigger difference in maternal body weight between the HF and CT groups). A second factor could be the age difference when the exposure to the high-fat diet started (a 4-week difference).

Here we report that a maternal high-fat diet alters hippocampal and cortical neurogenesis, and neuronal differentiation in E17 mouse fetal brains. Interestingly, we report opposite changes in the hippocampal and cortical V2/SVZ areas (increased proliferation of neural progenitors) versus decreased cell proliferation within the DG area. These changes associated with decreased apoptosis within selected hippocampal areas (Ammon’s horn and fimbria), and decreased calretinin levels within DG, the later indicating decreased early neuronal differentiation.

Since the peak of hippocampal and cortical cell proliferation is between embryonic days E13 and E16 (Rodier, 1977; Rodier, 1980), followed by a decrease in cell proliferation, one could speculate that the increase in cell proliferation within the V2/SVZ areas of HF fetal brains represents developmental delay (earlier stage of development with increased proliferation). Because the neural progenitors in DG migrate from the hippocampal neuroepithelium, specifically from the dentate notch (Altman and Bayer, 1990b; Altman and Bayer, 1990a; Pleasure et al., 2000), the decreased cell proliferation within the DG in HF brains could also be interpreted as a second mark of hippocampal developmental delay. However, in the absence of a time-course examination of neurogenesis, the hypothesis of developmental delay should be taken into consideration cautiously.

In this study, apoptosis in HF fetal brains was decreased in specific hippocampal areas (Ammon’s horn, CA and Fimbria, Fi, Fig. 4). During fetal brain development, apoptosis has been documented to be a contributing factor enabling the selection of appropriate cells before they complete their differentiation in postnatal life (Blaschke et al., 1996). In this context, decreased apoptosis reported here in the HF male fetal hippocampi could be interpreted as constitutive to developmental delay. However, other factors like psychological and pharmacological stressors have been implicated in decreased apoptosis within the CA hippocampal region (reviewed in (Lucassen et al., 2006)).

Decreased calretinin protein levels within the DG of HF fetal brains could be related to reduced early neuronal maturation. In both fetal and adult brains, calretinin is a transient marker for newly generated neurons and, thus, is considered a marker for early postmitotic neuronal differentiation (Soriano et al., 1994; Brandt et al., 2003).

This study has important limitations. The highly unbalanced diet containing 60% calories from saturated fat mainly is less representative for the obesogenic diets in humans (35% energy...
from fat (Miller et al., 1990)), and further animal studies should validate whether the alterations reported here are still present in inducing the described outcomes.

The relevance of rodent studies to the human epidemiological data on the relationship between maternal obesity, or high-fat diets uptake, and fetal outcomes is yet unclear. A recent meta-analysis indicated that, overall, obese mothers are at increased risk of having babies with various birth defects, including spina bifida, cardiovascular anomalies, cleft lip and palate, anorectal atresia, hydrocephaly, and limb reduction anomalies (Stothard et al., 2009). Two epidemiological studies indicated that obese mothers are also at increased risk of having babies that are either large (LGA) or small for gestational age (SGA). In a prospective study, nulliparous women having singleton babies were at increased risk of having SGA or LGA babies (Rajasingam et al., 2009). Although the results of this referenced study were in contrast with a previous study from the Swedish Birth Registry, which reported that obesity in nulliparous women protected against fetal growth restriction (Cnattingius et al., 1998), a second study in Chinese overweight women indicated that women who had BMI > 23 were at increased risk of having an SGA baby (Leung et al., 2008). Maternal overweight and obesity increased both the risk for fetal growth restriction and sudden intrauterine unexplained death (Froen et al., 2004).

In mouse models, different research groups reported discordant results on the relationship between maternal obesity and fetal growth, even when using the same mouse strain (C57Bl/6). When 4-week old females were fed a high-fat diet (57% calories from fat) for 6 weeks until mating and during pregnancy, no differences in body weight were reported at gestational day 18 and at birth, between the male pups from overweight mouse females and controls (Tozuka et al., 2009). Meanwhile, an independent study using 8-wk old females exposed to a high-fat diet (32% energy from fat), reported 43% higher fetal weights at E18.5 in the high-fat-fed group than in the control group, although there were no statistically significant differences in maternal weight at the time of mating (Jones et al., 2009). In an earlier study, Samuelsson et al. reported no changes in weight at birth, when 3-week old females were exposed to a high-fat diet 6 weeks prior, and during gestation (Samuelsson et al., 2008). Interestingly, the first two studies mentioned above (Jones et al., 2009; Tozuka et al., 2009) reported opposite outcomes regarding the relationship between maternal insulin resistance and fetal growth, and in opposition to the classical paradigm (i.e. maternal diabetes associates with increased birth weight). While Tozuka et al. reported increased insulin resistance associated with no changes in birth weight of pups (Tozuka et al., 2009), Jones et al reported no changes in maternal insulin sensitivity, yet bigger pups at birth (Jones et al., 2009). Potential causes for such different outcomes may include the age of pregnant mice and the duration of dietary treatment, the difference in fat composition and amount, suggesting that different fetal outcomes may be induced by different windows of opportunity for maternal exposures.

Whether the described alterations in hippocampal neurogenesis are transient or not is subject of further research. A previous study indicated that such changes may be long-lasting until postnatal day 70 (Tozuka et al., 2009).

Several mechanisms could account for the described outcomes. Alterations in hippocampal neurogenesis were related to peroxidized lipid accumulation in the dentate gyrus (Tozuka et al., 2009). Other studies indicated that maternal supplementation with n-6 PUFA led to significantly greater protein kinase C (PKC) activity in the hypothalamus and moderately less PKC activity in the whole brain of mouse offspring (Raygada et al., 1998). When on a high fat, dextrose rich diet, adult male rats and mice have decreased brain-derived neurotrophic factor (Bdnf) levels in the cortex and hippocampus (Yu et al., 2009), associated with alterations in discrimination reversal (Kanoski et al., 2007) and with reduced hippocampal spatial learning performance (Molteni et al., 2002). Another possible mechanism involved in the dietary regulation of hippocampal development is related to the leptin receptor, which is expressed in hippocampus (Louis and Myers, 2007), and may have an important role in facilitating memory and learning (Oomura et al., 2006). Obesity reduces the expression of leptin receptor in liver and hypothalamus (Liu et al., 2007), while lean mice over-expressed it in the dentate gyrus of the hippocampus (Lin and Huang, 1997).

In conclusion, we report, for the first time to our knowledge, that prenatal and gestational exposure to a high-fat diet induces SGA status which associates with fetal hippocampal development alterations at embryonic day 17, as determined by markers for cell proliferation, apoptosis, and early neuronal differentiation. The alterations in cell proliferation within the two main neuronal proliferation areas (VZ/SVZ and DG) are opposite and follow the pattern of hippocampal development, while apoptotic changes are confined to the Ammon’s horn and Fimbria. These outcomes associate with decreased early neuronal differentiation within the fetal dentate gyrus. Further studies are warranted to determine the mechanisms responsible for these outcomes.

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References
