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Maternal Adiposity Negatively Influences Infant Brain White Matter Development

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Abstract

Objective—To study potential effects of maternal body composition on central nervous system (CNS) development of newborn infants.

Methods—Diffusion tensor imaging (DTI) was used to evaluate brain white matter development in 2 week old, full-term, appropriate for gestational age (AGA) infants from uncomplicated pregnancies of normal-weight (BMI<25 at conception) or obese (BMI ≥ 30 at conception) and otherwise healthy mothers. Tract-based spatial statistics (TBSS) analyses were used for voxel-wise group comparison of fractional anisotropy (FA), a sensitive measure of white matter integrity. DNA methylation analyses of umbilical cord tissue focused on genes known to be important in CNS development were also performed.

Results—Newborns from obese women had significantly lower FA values in multiple white matter regions than those born of normal-weight mothers. Global and regional FA values negatively correlated ($P<0.05$) with maternal fat mass percentage. Linear regression analysis followed by gene ontology enrichment showed that methylation status of 68 CpG sites representing 57 genes with GO terms related to CNS development was significantly associated with maternal adiposity status.

Conclusions—These results suggest a negative association between maternal adiposity and white matter development in offspring.

Keywords

brain white matter; brain development; maternal adiposity; gestational fat mass; infant brain development

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TMB, AA, KS, and XO conceived the study. XO and KMT carried out experiments and analyzed data. All authors were involved in writing of this paper and have approved the final version.

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Introduction

Childhood obesity continues to be one of the most prominent pediatric health concerns in the US (1). While the etiology of childhood obesity is multifactorial, maternal adiposity is a significant risk factor for childhood obesity (2, 3). Results from experimental models and clinical studies in obese mothers suggest an important role of maternal programming of offspring metabolism (4, 5, 6, 7). Interestingly, recent studies also revealed an association between maternal obesity and long-term cognitive and neurodevelopment of the offspring (8, 9, 10, 11). Furthermore, in healthy children, BMI has been negatively linked to brain structure and function (12, 13). However, very little information is available concerning the influence of maternal adiposity on *in utero* brain development.

Diffusion tensor imaging (DTI) by magnetic resonance imaging (MRI) provides an extremely sensitive measure of brain white matter integrity. Studies using DTI have detected significant white matter microstructural differences in healthy children fed different infant diets (14), as well as significant positive correlations between greater white matter development and higher intelligence quotient (IQ) scores in the normal pediatric population (14, 15). Moreover, tract-based spatial statistics (TBSS), a new and objective DTI data analysis methodology to evaluate whole brain white matter (16), has revealed subtle effects of different ventilation strategies (17) or previously undetected hemorrhage (18) in premature infants.

Here we examine the hypothesis that maternal adiposity has unfavorable effects on white matter development in newborn infants. We recruited women with uncomplicated singleton pregnancies who were either normal-weight or obese at conception (based on BMI) and studied their healthy newborns at age 2 weeks using DTI. To further understand if changes in white matter development are associated with epigenetic alterations in genes involved in nervous system development, we assessed genome-wide DNA methylation of umbilical cord (UC) tissue of normal-weight and overweight/obese mothers. UC tissue derived from the extra-embryonic membranes is reflective of the fetal exposure following placentation and stem cells isolated from the Wharton's jelly (UC matrix) can differentiate into neurons or oligodendrocytes (19, 20, 21). Gene expression and epigenetic signatures in the UC have been previously shown to be influenced by maternal habitus and were used as a surrogate for examining the offspring epigenome.

Methods

Study population

The study population consisted of two week old, full-term, appropriate for gestational age (AGA) infants from uncomplicated pregnancies. The study protocol, including all procedures, was approved by the Institutional Review Board at the University of Arkansas for Medical Sciences (UAMS). Written informed consent was obtained from all participants. Subjects were enrolled in an ongoing longitudinal study of normal-weight and overweight/obese pregnant women, and their term infants (ClinicalTrials.gov ID: NCT01131117). All women recruited into the study were non-smoking mothers without pre-existing or existing gestational diabetes, pre-eclampsia or other pregnancy complications. All mothers were

second parity singleton pregnancies conceived without fertility treatments, and had their body composition assessed using air displacement plethysmography (Bodpod, Cosmed, Chicago, IL) and BMI measured within the first 10 weeks of gestation. Maternal IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI, Pearson, San Antonio, TX). Upon delivery, birth weight and length were retrieved from medical records; whereas, head circumference was measured at age 2 weeks. In a subset of subjects, umbilical cord (UC) was collected and stored in -70°C within 30 min of delivery. For infant brain structure studies, newborns of normal-weight ($\text{BMI} < 25$ at conception) or obese ($\text{BMI} \geq 30$ at conception) and otherwise healthy mothers were recruited. Table 1 summarized the demographic information of the mothers and infants who completed the study. For DNA methylation analysis we studied participants for whom UC was available. Table 2 summarizes the demographic information of these participants.

MRI examination

At approximately two weeks of age, MRI examinations of the infants' brain were performed in the Department of Radiology of the Arkansas Children's Hospital. They were fed ~ 30 minutes prior to the scan, swaddled in warm sheets, and immobilized using a MedVac Infant Immobilizer (CFI Medical Solutions, Fenton, MI). No sedation was used. A pulse oximeter probe (InVivo Corp, Florida, US) was placed on a foot to monitor oxygen saturation and heart rate, and mini-muffs were placed over the ears. The MRI examinations were performed on a 1.5 Tesla Achieva MRI scanner (Philips Healthcare, Best, the Netherlands) with 60 cm bore size, 33 mT/m gradient amplitude, and 100 mT/m/ms maximum slew rate. A pediatric 8-channel SENSE head coil was used. A MRI compatible camera was attached to the head coil and connected to a LCD in the control room to monitor the infants. A conventional neonatal brain MRI protocol was used for the investigators to exclude subjects with apparent brain abnormalities. In addition, a single shot spin echo planar imaging sequence with acquisition voxel size $2\text{ mm} \times 2\text{ mm} \times 3\text{ mm}$ and diffusion weighting gradients ($b = 700\text{ s/mm}^2$) uniformly distributed in 15 directions was used to acquire the DTI data. The imaging quality was reviewed on the scanner to exclude subjects with motion artifacts on DTI.

DTI TBSS analyses

The fractional anisotropy (FA) maps for each subject were computed from the scanner-carried software (Fibertrak) and were exported to a workstation with FMRIB Software Library (FSL, created by the Analysis Group, FMRIB, Oxford, UK) for TBSS analysis. The FA images were first preprocessed. FA data sets were aligned to identify the most representative one (which had the minimal amount of total warping) that consequently served as the target, and then nonlinear transforms were performed to register each FA data set to this target. Spatial normalization of FA data sets to existing brain templates (such as the MNI152 standard space) was not performed because of the large differences between neonatal brain in this study and adult brain in existing databases. Instead, the target FA data set served as a customized template in this study. Afterwards, all FA images were merged, averaged, and entered into the FA skeletonisation program in FSL to create a mean FA skeleton in which a threshold of $\text{FA} \geq 0.15$ was chosen. The registration and the skeletonisation were reviewed slice by slice to ensure no apparent artifacts. Finally, the

randomization program in FSL was used to perform comparison analysis of FA values between the maternally normal-weight and obese groups and correlation analysis of FA values and maternal fat mass percentage. The maternal fat mass percentage was used in the correlation analysis because the fat mass percentage is a more accurate measurement of maternal adiposity than BMI. Mean FA values of whole brain white matter and in several important white matter regions which showed significant differences in TBSS analysis were also calculated for each infant and were compared between maternally normal-weight/obese as well as correlated with maternal fat mass percentage.

Statistical analysis for DTI studies

For the comparison of demographic parameters between the maternally normal-weight and obese groups, Wilcoxon rank-sum tests were performed by Matlab software (The MathWorks, Inc. Massachusetts, US) to determine if there were significant differences ($P < 0.05$). For the correlation between whole brain and regional white matter mean FA and maternal fat mass percentage, Spearman's rank partial correlation coefficient was calculated by Matlab, and $P < 0.05$ after controlling for gestational weight gain, infants' gender, birth weight, length, head circumferences, diet, and postmenstrual age at MRI (sum of gestational age and age at MRI) was regarded significant. Partial correlation between mean FA and the demographic parameters were also performed. For the DTI TBSS analyses, randomization with the threshold-Free Cluster Enhancement (TFCE) option and 5000 permutations was used for both the voxel-wise comparison between the maternally normal-weight and obese groups and the voxel-wise correlation between infant FA and maternal fat mass percentage. To correct for multiple comparisons, the observed TFCE image was compared to the empirical null distribution computed across permutations of the maximum voxels-specific TFCE scores (16, 22). The analyses were also adjusted for gestational weight gain, infants' gender, birth weight, length, head circumference, diet, and postmenstrual age at MRI, which were included as covariates during the randomization.

Genome-wide DNA methylation analysis

UC genomic DNA was isolated from 100 mg of tissue using the Wizard Genomic DNA purification kit (Promega, Madison, WI) (23). UC genomic DNA (1 μ g) was bisulfite converted using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA). Genome-wide DNA methylation was assessed using the Infinium HumanMethylation450 (HM450K) BeadChip array (Illumina, San Diego, CA). Hybridization, staining and scanning steps for all samples were performed at the same time to avoid batch effects using the standard Infinium HD assay Methylation Protocol guide. Imaging was carried out using the Illumina iScan and resulting files were used to extract methylation values for each probe as described below. Preliminary quality assessment was done using GenomeStudio Methylation module (Illumina) for bisulfite conversion and for staining, hybridization, extension and specificity. All further steps including data import, normalization, filtering and analyses were carried out in the R environment using packages implemented in the *ChAMP* pipeline(24). Data import and quality control were performed from IDAT files using functions included in the *minfi* package. Probes with detection P -values > 0.01 and those on the X and Y-chromosomes were excluded. Normalizations between type I and type II probes (type 2-bias) were performed using the beta-mixture quantile normalization (BMIQ) method.

Percent methylation values for each CpG site (β -values) and log2-transformed ratios of methylated to unmethylated probe intensities (M-values) were extracted for further analysis.

Statistical analysis for HM450K arrays

To examine the effects of maternal fat mass, a mixed model regression framework was utilized with the *MethLAB* package in R (25). We modeled methylation data (β -values) at each site against maternal fat mass as a continuous variable. Covariates included gestational weight gain, maternal age, infant sex, and birth weight. *P*-values were adjusted for multiple testing by controlling false discovery rate (Benjamini-Hochberg). Differentially methylated CpG sites (DMS) were annotated with gene symbols and genomic location and features using HM450K annotation provided by Illumina. Absolute difference in methylation (Δ me) and percent change in methylation over control (% Δ me) was calculated using β -values. Enrichment of gene ontology terms was performed in GOrilla using the entire list of genes present on the HM450 array as background. Identification of enrichment of KEGG pathways, OMIM disease terms, and transcription-factor binding motifs were carried out using DAVID. The BiNGO plugin in Cytoscape was used to visualize GO terms. Circular plots for representing genome-wide methylation data were generated using *RCircos 2* package.

In addition, further methylation analysis using Bisulfite amplicon sequencing (BSAS) of UC genomic DNA, and UC RNA isolation and Real-time RT-PCR analysis were performed (see SUPPLEMENT).

Results

Twenty-eight infants (normal-weight, maternal BMI<25 at conception, N=17; obese, maternal BMI $\geq$ 30 at conception, N=11) completed the DTI studies. Maternal BMI and fat mass percentage were significantly greater in the obese group; whereas, maternal IQ did not differ between groups (Table 1). Infants from both groups had similar birth weight, birth length, head circumference, and postmenstrual age at MRI.

Voxel-wise comparison of FA values showed widespread white matter tracts with significantly higher FA ($P<0.05$, higher FA represents better development) in the maternally normal-weight group, after correcting for multiple comparisons and adjusting for gestational weight gain, infants' gender, birth weight, length, head circumference, diet, and postmenstrual age at MRI (Figure 1). These tracts involved association, projection, callosal, and limbic fibers including the inferior fronto-occipital longitudinal fasciculus, the superior longitudinal fasciculus, the external and anterior internal capsule, the forceps minor, the genu of the corpus callosum, the fornix, and the anterior and superior corona radiata. In addition, voxel-wise correlation analysis of infant FA and maternal fat mass percentage showed significant negative correlation ($P<0.05$, fully corrected) in widespread white matter tracts after adjusting for gestational weight gain, infants' gender, birth weight, length, head circumference, diet, and postmenstrual age at MRI (Figure 2). These tracts are very similar to the tracts which differed significantly between the maternally normal-weight/obese groups, with the addition of the body and splenium of corpus callosum. No region/tract/voxel showed higher FA in the maternally obese group, or positive correlation between

infant FA and maternal fat mass percentage. The infant voxel-wise FA did not correlate with maternal IQ, infant birth weight/length, head circumference, or gestational weight gain. However, as expected, the infant's FA values positively correlated with postmenstrual age at MRI in all major white matter regions (see supplemental Figure S1) because the brain develops rapidly during neonatal period. Furthermore, the global measure of whole brain white matter mean FA values and most regional mean FA values significantly differed between the maternally normal-weight or obese groups, and the mean FA values for the whole brain or for major white matter regions each significantly and negatively correlated with maternal fat mass percentage, after adjusting for all covariates (Figure 3).

Umbilical cord (UC) was used as a surrogate for fetal tissue and studied for DNA methylation to gain potential mechanistic insights. Maternal BMI and fat mass percentage were significantly greater in the maternally overweight/obese group than the normal-weight group, while other parameters did not differ (Table 2). Using the HumanMethylation450K array (Illumina), we screened CpG methylation in UC and conducted epigenome-wide association analysis for maternal adiposity. DNA methylation of 2,966 CpG sites in UC was significantly associated with maternal adiposity (FDR corrected $P < 0.05$). Gene ontology analysis of the differentially methylated CpG sites (DMS) revealed that maternal adiposity affected methylation of genes primarily involved in cellular metabolism, apoptosis, stress response and organ and tissue development, including brain development (Supplemental Figure S2). Among these, we identified 57 genes with GO terms related to nervous system development that differentially methylated based on maternal obesity status (Figure 4A-B, See Supplemental Figure S3 for Legend for the Circos diagram and Supplemental Table S2 for gene locations). These genes had bona fide roles in neurogenesis, neuron differentiation, axonogenesis, and particularly white matter development. Hierarchical clustering revealed a sub-set of genes that showed distinct hypermethylation in association with maternal obesity (Figure 4C). We identified CpGs with strong positive (*NKX2-1*, *LMX1A*, *FOXA2*, *HES1*, *DAB1* and *BCL2*) and negative (*GSX2*, *IGF-1R* and *FYN*) association with maternal fat mass (Figure 4D). Further methylation analysis was performed using BSAS of key neuronal development-related genes identified from the HM450K methylation array that both positively and negatively correlated with maternal fat mass (see Supplement).

Discussion

Maternal obesity during pregnancy is associated with programming of offspring development and metabolism, and recent studies have uncovered an association between maternal obesity and offspring neurodevelopment. In the current study, we examined the relationship between maternal adiposity and white matter development in newborn infants. Using DTI, we observed that offspring white matter development inversely correlates with maternal adiposity. Moreover, global DNA methylation analysis of offspring UC at birth identified several nervous system development genes that were differentially methylated based on maternal obesity status. These data suggest that maternal obesity is associated with offspring white matter development and epigenetic alterations in genes involved in neurodevelopment. Our findings of differential white matter integrity in newborns from normal-weight and obese mothers and the observed correlation between maternal fat mass

and DNA methylation of UC genes involved in brain development are the first to demonstrate these changes may be programmed *in utero*.

Neurodevelopmental studies suggest associations between maternal obesity and reduced cognitive development (9) and delayed mental development in infants (10) which are likely to persist into later ages. For example, maternal pre-pregnancy BMI was negatively associated with children's cognitive performance at age 5 and 7 years (8), and was associated with increased risk of inattention symptoms and difficulty with emotion intensity and regulation in kindergarten children (11). The molecular mechanisms that link maternal obesity and neurodevelopment are still under investigation (30). Recent experimental studies demonstrated that maternal adiposity affects offspring hippocampal and hypothalamic inflammation (26, 27), orexigenic and anorexigenic signaling (28), and brain reward systems (29). Likewise, a recent report examining cell-free transcriptomic signatures in 2nd trimester amniotic fluid between normal-weight and obese women found the greatest effects in neurodevelopmental and metabolic genes, including increased apolipoprotein D and *BCL2* mRNA expression (30). Along these lines, we observed in the HM450K array that gene-body methylation of *BCL2* was increased in relation to maternal fat mass. Furthermore, in the 10 overlapping subjects who completed both the MRI study and the epigenetics study, significant negative association was found ($r=-0.63$, $P<0.05$) between *BCL2* methylation and DTI-measured FA values. Many of the other genes we identified from the global HM450k DNA methylation array (eg. *NKX2-1*, *LMX1A*, *FOXA2*, *HES1*, *DAB1*, *FYN*, *GSL2* and *IGF1R*) play critical roles in oligodendrocyte and oligodendrocyte progenitor cell differentiation and thus myelination in white matter. Our data suggest that alterations in UC DNA methylation may reflect fetal white matter development, as reflected by our DTI measurements at birth. This has potential long-term implications, since white matter injury during infancy has been shown to result in adverse neurodevelopmental outcomes (31), and white matter abnormalities have been reported in numerous neurodevelopmental disorders in children.

The coexistence of obesity and brain abnormalities has been previously reported (32) and studies have shown associations between obesity and differences in brain structures, such as decreased total brain volume (33) and total grey matter volume (34), decreased white matter integrity (35), and atrophy in multiple brain regions (36). However, it is not clear whether there is a causal effect, i.e., whether brain abnormalities are indeed a cause or a consequence of obesity. In the current study, infants born of normal-weight and obese women were all AGA and the two groups did not differ significantly in birth weight or other notable measures at birth. However, significant white matter integrity differences were present at age 2 weeks, suggesting that some pre-conception or *in utero* factor(s) or event(s) in obese women inhibited white matter development in their offspring compared with offspring from leaner women. Our findings therefore suggest that detectable structural differences in the brain precede childhood obesity and are a consequence of maternal obesity.

The observed white matter differences between maternally normal-weight or obese groups and negative correlations between white matter integrity and maternal fat mass percentage were mainly located in the anterior, but not posterior brain. While the specific reasons are unknown, they may be related with the developmental pattern of infants brain, since

myelination and maturation of white matter usually start from posterior then anterior brain, or related with selective brain functions that are most impacted by programming of maternal obesity. In our study, the anterior brain including the frontal lobe which is important for cognitive functioning showed maternal obesity associated differences, but not the major motor pathway such as the posterior limb of internal capsule or the major visual pathway such as the optic radiation.

In summary, results from this study suggest both global and region-specific negative correlation between FA and maternal fat mass percentage. Since FA is a known reflection of white matter integrity, these findings further suggest that maternal adiposity is associated with lower white matter integrity in full-term AGA newborn infants than similar infants of normal-weight mothers. Because these infants had not yet been exposed to confounding environmental factors, such as long-term diet or nutritional status deficiencies, it is likely this association was primarily influenced by *in utero* factor(s). Significant association between maternal fat mass and UC DNA methylation of specific genes which are involved in CNS development and function revealed evidence of maternal programming. These data are consistent with the concept of maternal programming of fetal metabolism which results in reduced brain white matter development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is already known about this subject

- Maternal obesity is associated with increased risk of childhood obesity
- Negative association between maternal pre-pregnancy BMI and offspring neurodevelopmental outcome has been observed

What this study adds

- Pre-pregnancy maternal adiposity is associated with lower in utero brain white matter development.
- Association is found between maternal fat mass and DNA methylation of genes known to be involved in CNS development

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Figure 1. Voxel-wise TBSS analysis showed that offspring from normal-weight mothers had widespread white matter tracts with higher FA values than maternally obese counterparts ($P < 0.05$, after correction for multiple comparisons and adjusting all covariates) Difference maps are presented here. The mean skeleton of major white matter tracts (green) for all infants is overlaid on raw DTI images (the background, 15 axial slices of one representative infant are illustrated here). Orange/yellow color on the skeleton represents regions with significantly higher FA in the maternally normal-weight group.

Figure 2. Voxel-wise TBSS analysis showed that FA values in widespread white matter tracts in infants negatively correlated with their maternal fat mass percentage ($P < 0.05$, after correction for multiple comparisons and adjusting all covariates)

Orange/yellow color represents regions with significant negative correlation. Note these regions are greatly consistent with those in Figure 1. There were no voxels with significant positive correlation.

Figure 3.

Mean FA values significantly differed ($P < 0.05$) between the maternally normal-weight and obese groups in whole brain white matter and in most of the studied white matter regions (bar graphs). The FA values in whole brain white matter and in all of the studied white matter regions significantly and negatively correlated with maternal fat mass percentage (scatter plots): whole brain (WB, $r = -0.51$, $P = 0.02$); genu of corpus callosum (GCC, $r = -0.58$, $P < 0.01$); inferior frontal-occipital fasciculus (IFO, $r = -0.57$, $P < 0.01$); superior longitudinal fasciculus (SLF, $r = -0.57$, $P < 0.01$); anterior limb of internal capsule (ALIC, $r = -0.43$, $P = 0.05$); external capsule (EC, $r = -0.50$, $P = 0.02$); body of corpus callosum (BCC, $r = -0.71$, $P < 0.01$); and superior corona radiata (SCR, $r = -0.51$, $P = 0.02$).

Figure 4. Umbilical cord DNA methylation analysis reveals differential methylation of neurodevelopmental genes

Genome-wide DNA methylation of UC was assessed using Infinium HM450 BeadChip from women based on maternal fat mass percentage. Differentially methylated sites (DMS) were identified via linear regression of β -values against maternal fat mass percentage ($P < 0.05$) using MethLAB. (A) Circos diagram showing DNA methylation changes associated with maternal adiposity. A detailed legend is included in Supplementary Figure S3. Tracks from outside to inside represent; *track 1* chromosomes; *track 2*, karyotype; *track 3*, location of 68 CpG sites relating to neurodevelopment whose methylation status is associated with maternal fat mass. Red represents hypermethylation and blue represents hypomethylation due to maternal obesity; *track 4*, gene symbol; *track 5*, average % methylation at the site (inner lines represent % methylation from 0 to 100 in increments of 20); *track 6*, average difference in methylation between maternally normal-weight and overweight/obese groups (me); *track 7*, correlation between maternal fat mass percentage and %methylation at the CpG site. (B) GO biological process terms relating to neuronal development enriched in sites associated with maternal adiposity. (C) Hierarchical clustering of selected CpG sites showing distinct hypermethylation in maternally overweight/obese subjects. (D) Association of % methylation of genes with strong roles in axonogenesis and oligodendrocyte maintenance and differentiation with maternal fat mass percentages.

Table 1
Demographic parameters of normal-weight (BMI<25) and obese (BMI ≥ 30) mothers and their newborn offspring who completed the DTI examination

Data are presented as mean±SD, and significant group differences (P<0.05) are highlighted in bold.

	normal-weight	obese	P value
N	17	11	
Maternal age (yr)	27.7±2.9	30.1±5.5	0.15
Maternal BMI (kg/m ²)	22.1±1.7	33.1±1.8	<0.01
Maternal fat mass (%)	29.9±4.0	44.0±4.6	<0.01
Maternal IQ	108.5±6.8	105.7±8.6	0.24
Gestational weight gain (Kg)	13.3±1.5	8.0±3.0	<0.01
Gestational age (weeks)	39.1±0.9	39.0±0.8	0.78
Infants' gender (boy/girl)	12/5	6/5	0.39
Birth weight (Kg)	3.5±0.4	3.8±0.5	0.11
Birth length (cm)	50.8±2.4	50.6±2.0	0.74
Head circumference (cm)	36.5±0.9	36.1±1.0	0.24
Age at MRI (weeks)	2.1±0.2	2.2±0.5	0.76
Postmenstrual age at MRI ^I	41.2±0.9	41.2±0.7	0.98
Infants' diet (breastmilk/formula/mix)	13/2/2	8/2/1	0.82

^I Postmenstrual age at MRI (weeks): sum of gestational age and age at MRI

Table 2
Demographic parameters of normal-weight (BMI<25) and overweight/obese (BMI ≥ 25) mothers and their offspring utilized for epigenetic analysis

Data are presented as mean±SD, and significant group differences (P<0.05) are highlighted in bold.

	normal-weight	overweight/obese	P value
N	12	24	
Maternal age (yr)	29.7±0.6	30.3±0.6	0.50
Maternal BMI (kg/m ²)	20.9±0.4	29.4±0.7	<0.01
Maternal fat mass (%)	27.3±1.0	41.3±1.1	<0.01
Maternal IQ	107.7±7.0	104.1±6.3	0.13
Gestational weight gain (Kg)	11.7±0.7	11.6±1.0	0.97
C-section (yes/no)	3/9	11/13	0.22
Gestational age (weeks)	39.3±0.2	39.4±0.2	0.58
Infants' gender (boy/girl)	4/8	11/13	0.47
Birth weight (kg)	3.4±0.1	3.6±0.1	0.09
Birth length (cm)	50.8±0.6	51.5±0.4	0.27
Head circumference (cm)	36.1±1.0	36.1±1.3	0.93