Maternal prepregnancy body mass index and offspring white matter microstructure: results from three birth cohorts

Running title: Maternal body mass index and child white matter

List of authors and affiliations

Juan Verdejo-Román[1]*, Lassi Björnholm[2,3,4]*, Ryan L. Muetzel[5,6,7], Francisco José Torres-Espínola[8,9], Johannes Lieslehto[2,3,4], Vincent Jaddoe [6,10], Daniel Campos[8,9], Juha Veijola[2,3,4], Tonya White[5], Andrés Catena[1], Juha Nikkinen[4,11], Vesa Kiviniemi[12], Marjo-Riitta Järvelin[13], Henning Tiemeier[5,14], Cristina Campoy[8,9], Sylvain Sebert[13], Hanan El Marroun[5,6,10,15]

* These authors contributed equally to this work

1. Mind, Brain and Behavior Research Center (CIMCYC), University of Granada, Spain
2. The Department of Psychiatry, Research Unit of Clinical Neuroscience, University of Oulu, Oulu, Finland
3. Department of Psychiatry, Oulu University Hospital, Oulu, Finland
4. Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Finland
5. The Department of Child and Adolescent Psychiatry, Erasmus MC, Sophia Children's Hospital, Rotterdam, 3000 CB, The Netherlands
6. The Generation R Study Group, Erasmus MC, Rotterdam, 3000 CA, The Netherlands
7. The Department of Epidemiology, Erasmus MC, 3000 CA, The Netherlands
8. EURISTIKOS, Excellence Center for Pediatric Research, University of Granada, Spain
9. The Department of Pediatrics, School of Medicine. University of Granada, Spain

10. The Department of Pediatrics, Erasmus MC, Sophia Children's Hospital, Rotterdam, 3000 CB, The Netherlands
11. Department of Oncology and Radiotherapy, Oulu University Hospital, Finland
12. Institute of Diagnostics, Department of Diagnostic Radiology, Oulu University Hospital, Oulu, Finland
13. Center for Life Course Health Research, University of Oulu, Oulu, Finland
14. The Department of Social and Behavioral Science, Harvard TH Chan School of Public Health, Boston, USA
15. Department of Psychology, Education & Child Studies, Erasmus University Rotterdam, The Netherlands

Corresponding Author: Dr. Hanan El Marroun, Department of Child and Adolescent Psychiatry, Department of Pediatrics, Erasmus MC- Sophia Children's Hospital, PO.Box 2060, 3000 CB, Rotterdam, The Netherlands. E-mail: h.marrounel@erasmusmc.nl

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University, and the Netherlands Organization for Health Research and Development (ZonMW, grant ZonMW Geestkracht 10.000.1003).

Abstract

Background and Aims: Prepregnancy maternal obesity is a global health problem and has been associated with offspring metabolic and mental ill-health. However, there is a knowledge gap in understanding potential neurobiological factors. This study explored the relation between maternal prepregnancy body mass index (BMI) and offspring brain white matter microstructure at the age of 6, 10 and 26 years in three independent cohorts.

Subjects and Methods: The study used data from three European birth cohorts (n=116 children aged 6 years, n=2466 children aged 10 years, and n=437 young adults aged 26 years). Information on maternal prepregnancy BMI was measured before or during pregnancy and offspring brain white matter microstructure was measured at age 6, 10 or 26 years. Magnetic resonance imaging derived fractional anisotropy (FA) and mean diffusivity (MD) were used as measures of white matter microstructure in the brainstem, callosal, limbic, association and projection tracts. Linear regressions were fitted to examine the association of maternal BMI and offspring white matter microstructure, adjusting for several socioeconomic and lifestyle-related confounders, including education, smoking and alcohol use.

Results: Maternal BMI was associated with higher FA and lower MD in multiple brain tracts, for example association and projection fibers, in offspring aged 10 and 26 years, but not at 6 years. In each cohort maternal BMI was related to different white matter tract and thus no common associations across cohorts were found.

Conclusions: Maternal BMI was associated with higher FA and lower MD in multiple brain tracts in offspring aged 10 and 26 years, but not at 6 years of age. Future longitudinal studies should examine whether these associations persist in later stages of development and explore the causal nature of the findings.

Introduction

Maternal obesity is a worldwide public health problem that has been linked to multiple health consequences affecting the mother and her offspring. Studies have investigated the association between prepregnancy maternal obesity and subsequent increased risk of child obesity (1), diabetes (2) and cardiovascular events in adult life (3). In addition, maternal obesity has been associated with adverse neurodevelopmental outcomes in offspring including lower intelligence and cognitive functioning (4-9). Maternal body mass index (BMI) has also been related to other neurodevelopmental outcomes, including lower performance in fine motor skills (10), executive functioning (11), attention problems, negative emotionality (12), and externalizing problems (13). This is also supported by a recent systematic review reporting evidence for an association between prepregnancy maternal obesity and several neurodevelopmental factors including cognitive and motor abilities in children (14).

Together, these findings suggest that fetal exposure to maternal obesity may influence offspring neurodevelopment with long-term metabolic and mental consequences, though the underlying mechanisms have yet to be elucidated. For this purpose, neuroimaging techniques can be used to better understand the possible associations between maternal obesity on offspring brain structure and function. Recently, it has been shown that maternal obesity was negatively associated to structural and functional brain connectivity in neonates (15, 16). In addition, newborns of mothers with obesity had lower fractional anisotropy (FA) in several white matter tracts, including projection, association, callosal, thalamic and limbic system fibers when compared to controls (16). Furthermore, exposure to maternal obesity was related to differences in resting-state functional connectivity in the dorsal anterior cingulate cortex (i.e. a brain region that is connected with the prefrontal and parietal cortex) in newborns (15). These two studies

were performed in a small group of newborns (n<40), and thus the long-term consequences of maternal obesity on brain development in childhood and adulthood remain unanswered.

The current study aimed to investigate the association of maternal pre-pregnancy BMI and offspring white matter microstructure at the age of 6, 10 and 26 years using three prospective birth cohorts. In the absence of longitudinal data, we used three cohorts with participants of different ages ranging from childhood to young adulthood to address the research question. Based on the prior work in neonates (16), we hypothesized that maternal prepregnancy BMI is associated with widespread differences in white matter microstructure. Given the sparseness of the literature, an exploratory approach covering a set of 13 major white matter tracts was chosen to study the association between prepregnancy BMI and offspring white matter microstructure.

Methods

The present study consists of participants drawn from three birth cohorts, including the PREOBE Study from Granada, Spain, the Generation R Study from Rotterdam, Netherlands, and the Northern Finland Birth Cohort 1986 (NFBC 1986), from the Northern Finland. Detailed information about inclusion and exclusion criteria for each cohort is included in the supplementary material. All studies were approved by their local Medical Ethics Committee.

Setting & participants

The PREOBE *Study*

The PREOBE study (17) was designed as a prospective observational cohort study exploring peri- and postnatal influences of maternal weight status on the offspring. Of the 331 mothers included in the study, 135 gave consent for neuroimaging of the offspring at 6 years old. 19 of

the 135 participants were discarded due to motion artifacts during acquisition, or other scanner-related artifacts. A final sample of 116 was included in the analysis.

The Generation R Study

The Generation R Study (www.generationr.nl) is an ongoing population-based prospective cohort study in Rotterdam (the Netherlands) designed to identify early environmental and genetic determinants of health and disease from fetal life onwards (18, 19). At approximately 10 years of age, 3992 children visited the research center for the neuroimaging session. Of these children, 3063 children had usable DTI data, but in 587 children information on maternal prepregnancy BMI was missing. 10 children were excluded from the analyses as they had radiological incidental findings which could potentially influence the white matter tracts and their quality. Thus, the study population for analyses included 2466 children with information on maternal BMI and data of white matter microstructure.

The NFBC 1986 Study

The Northern Finland Birth Cohort 1986 Study (NFBC 1986; http://www.oulu.fi/nfbc/) is a prospective population-based data collection effort of health-related information on individuals with an expected date of birth between the 1st of July 1985 and the 30th of June 1986 in the two northernmost provinces of Finland. A total of 9 362 deliveries, i.e. 99% of all deliveries in the target period, were recorded in the cohort register (20). The 26-year subsample, used in the present study, was collected based on the participants of a 16-year follow-up. Owing to the original study question, almost 50% of the participants were exposed to maternal smoking during pregnancy. Of the invited 1396 eligible participants, a total of 471 (34 %) participated in the

study. Scanning was completed successfully in 451 participants (21). Common contraindications for the MRI acquisition included pregnancy, participant's metal or electronic implants and severe claustrophobia. Of the 451 participants with neuroimaging data, one was excluded due to large ventricles preventing image processing errors and three due to a failed MRI protocol. Also, 10 individuals had missing maternal BMI data, leaving altogether 437 individuals for the analysis.

Maternal BMI

In the PREOBE study, maternal height were measured at the recruiting session between week 12 and 20 of gestation. Prepregnancy maternal weight was self-reported at the same session. In the Generation R Study, information about weight just before pregnancy was obtained by questionnaire. At enrollment, we measured height (cm) and weight (kg) without shoes and heavy clothing. The correlation of prepregnancy weight obtained by questionnaire and weight measured at enrollment was 0.95 ($p < .001$). In the NFBC 1986 study, prepregnancy weight was reported by the mothers at visits to maternity health centers in the seventh or eighth month of pregnancy. Maternal height was measured in 52% of mother's during the same visit and self-reported by the rest (22). Information of maternal weight and height was used to calculate maternal prepregnancy BMI in kg/m2.

Neuroimaging

Image acquisition

Scanner characteristics and technical acquisition parameters from each cohort are reported in Table 1. Children in the PREOBE and Generation R cohort underwent a mock scanning session prior to the actual MRI scan session.

Preprocessing

All three cohorts used the same processing pipeline using the same software (23); DTI images were processed using the functional MRI of the Brain's software library (FMRIB, FSL, 24). Image processing included adjustment for minor head motion (translations and rotations) and eddy-current induced artifacts (25), rotation of the gradient direction table in the same way than the images in the previous step, and non-brain tissue removal using the FSL Brain Extraction Tool (26) and finally calculation of FA and MD maps by fitting the diffusion tensor using dtifit function (PREOBE and NFBC1986) or the RESTORE method implemented in Camino (Generation R). The quality of raw diffusion-weighted images was assessed using the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/) that automatically examined the data for slice-wise variation, a characteristic of artifact, in each diffusion-weighted volume. The sum-of-squares error (SSE) maps from the diffusion tensor calculations were examined for structured signal that was indicative of artifact. Each SSE map was rated from 0 to 3 (0: "None", 1: "Mild", 2: "Moderate", 3: "Severe"). Any cases not excluded by the automated DTIPrep tool that had a "Severe" score from the SSE rating were also excluded from analyses.

Probabilistic fiber tractography

The automated *AutoPtx* (27) pipeline was used to run probabilistic tractography for brainstem, projection, association, callosal, thalamic and limbic system fibers in each individual (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/AutoPtx). As part of the pipeline, native DTI data were registered to FMRIB-58 1-mm standard space and the alignment was visually inspected. Tracts were defined using seed, target, termination, and exclusion masks that were warped to native

diffusion space. After normalization of the resulting connectivity distributions by the total number of successful seed-to-target attempts for each tract, the connectivity distributions were thresholded (27). The resulting tract masks were visually inspected for misclassified voxels and then the white matter characteristics mean fractional anisotropy (FA) and mean diffusivity (MD) parameters per tract were extracted. Tracts that appeared in both the left and right hemisphere were averaged to reduce the number of tests. Likewise, the three 'thalamic radiation' tracts, namely the anterior, posterior and superior thalamic radiation, were averaged together, producing altogether 13 tracts of interest.

Potential confounders

Potential lifestyle and socioeconomic confounders were selected based in previous studies (28, 29). Maternal age, smoking and drinking habits during pregnancy, ethnicity and education were assessed using questionnaires. Offspring birth weight and sex were obtained from medical records. Age of the child, as well as height and weight, were assessed at the MRI visit.

Statistical Analyses

Descriptive information of each cohort was provided. Associations between maternal prepregnancy BMI (z-scores) and white matter microstructure (FA and MD) were tested using multiple linear regression models separately in each cohort. First, the unadjusted linear relation between maternal prepregnancy BMI and white matter microstructure for each tract was analyzed (these results can be found in the Supplemental Material). Subsequent models were adjusted for lifestyle and socioeconomic confounders including maternal age, smoking and drinking habits during pregnancy, maternal ethnicity, educational level, and birth weight, age and

sex of the child. The effect estimates (unstandardized B's) can be interpreted as the adjusted difference in FA or MD per change in one unit of maternal BMI in z-scores. P-values were adjusted for multiple comparisons using a false discovery rate (FDR) (30) correction for the 13 tracts in each cohort. An adjusted p-value less than 0.05 was considered significant.

The models were further adjusted with offspring height at time of the neuroimaging assessment, to ensure the associations were not confounded by size of the offspring. Additionally, in the largest cohort (the Generation R cohort) we also explored whether there were curvilinear (quadratic) associations of prepregnancy maternal BMI and offspring white matter microstructure to examine whether the undernutrition or obesity were driving the associations. All statistical analyses were carried out using the R Statistical Software, version 3.4.1. (31) and SPSS version 24 (Chicago, IL, USA).

Results

Sociodemographic, anthropometric and lifestyle characteristics of the three cohorts are reported in Table 2. In the PREOBE cohort, 22.4% of the mothers were overweight and 16.4% were obese before pregnancy (mean BMI 25.1). In the Generation R cohort, 24.5% of the women were overweight and 9.8% were obese before pregnancy (mean BMI 24.5), while in the NFBC 1986 cohort 11.4% and 5.7% were overweight or obese (mean BMI 22.5), respectively. While the PREOBE and Generation R cohort had a high percentage of higher educated women, the NFBC 1986 mostly consisted of participants with a secondary education. Finally, the three cohorts differed considerably in terms of alcohol use and smoking during pregnancy (Table 2).

Fractional Anisotropy

Table 3 shows the associations between maternal BMI and FA of the white matter tracts in each cohort. In the PREOBE cohort of children aged 6 years, we found no associations of maternal BMI and offspring FA. However, associations of maternal BMI with multiple tracts were found after correction for multiple comparisons in the children aged 10 years of the Generation R cohort and young adults aged 26 years in the NFBC 1986 cohort. More specifically, Table 3 shows that, in the Generation R cohort, maternal BMI was negatively associated with FA in the forceps minor and the medial lemniscus, while maternal BMI was positively associated with the middle cerebellar peduncle, the cingulate gyrus, the parahippocampal part of the cingulum, the inferior fronto-occipital fasciculus, the acoustic radiation and the thalamic radiation at age 10 years (Table 3). In the NFBC1986 sample, positive associations between maternal BMI and FA were found in the superior longitudinal fasciculus and the corticospinal tract at age 26 years (Table 3). For illustrative purposes Figure 1A visualizes the white matter tracts associated with maternal BMI in each cohort, and Figure 2A shows the effect estimates with their 95% confidence intervals in a graph.

No quadratic associations of maternal BMI and offspring FA were found. In addition, additional adjustment for offspring height did not change the results.

Mean Diffusivity

Table 4 show the associations between maternal BMI and MD of the white matter tracts in each cohort. Again, in the PREOBE cohort, we found no associations of maternal BMI and offspring MD at 6 years (Table 4). In the Generation R cohort, maternal BMI was related to lower MD in the parahippocampal part of the cingulum at 10 years. In NFBC 1986 maternal

BMI was associated with lower MD in multiple white matter tracts, including the medial lemniscus, the inferior longitudinal fasciculus, the uncinate fasciculus, the corticospinal tract and the thalamic radiation at 26 years (Table 4).

Figure 1B illustrates the white matter tracts in colors and Figure 2B the effect estimates with their 95% confidence intervals in a graph in each cohort.

Likewise, no quadratic associations of maternal BMI and offspring MD were found and additional adjustment for offspring height did not change the results.

Discussion

Main findings

The aim of the current study was to examine the association between maternal prepregnancy BMI and offspring white matter microstructure. We presented results obtained in three different European prospective birth cohorts. In this study, maternal BMI was associated with higher FA and lower MD in multiple brain tracts, for example association and projection fibres, in offspring aged 10 (the Generation R cohort) and 26 years (the NFBC 1986 study), but not at 6 years (the PREOBE cohort). In both cohorts, maternal BMI was related to different white matter tract and thus no common associations across cohorts were found.

Existing literature

Acknowledging possible publication bias, to our knowledge there is only one prior study that investigated the current association of maternal obesity and white matter microstructure in neonates, which complicates comparing our results with the existing literature. This prior case-control study demonstrated that maternal obesity was associated with lower FA in 2-week-olds

in association, projection, callosal and limbic white matter tracts (16). In contrast, in the current study we found associations of maternal BMI and higher FA in some of these white matter tracts in the Generation R cohort at age 10 years and the NFBC 1986 study at age 26 years, but not in the PREOBE cohort at age 6 years. This discrepancy may be explained by differences in study design and methodology, as well as the small sample size in the PREOBE cohort. In addition, as the participants in the three cohorts used in the current study are years older, it is possible that these differences are due to the enormous development of white matter (i.e. myelination) during the first years of life (32).

A study of newborns using resting-state functional MRI showed that maternal obesity was related to decreased connectivity in the dorsal anterior cingulate cortex (15). In addition, maternal obesity was associated to weaker self-regulatory responses to cues of food items in the dorsal anterior cingulate cortex in offspring (33). It is possible that our finding that maternal BMI was related to microstructure of the cingulum tract in children aged 10 years may relate to these earlier findings and thus suggest changes in connectivity of the limbic system (34).

Interpretation of the findings and explanations

Maternal BMI was associated with higher FA and lower MD in multiple brain tracts in offspring aged 10 and 26 years, but not at 6 years. However, effect estimates were small and none of these associations of maternal BMI and the individual white matter tracts were consistent between the two cohorts. We must be cautious with the interpretation of these results because cohort and MRI scanner effects cannot be distinguished from possible age effects due to cross-sectional study design even though the processing methodology and analysis was uniform across sites.

Several hypotheses have been proposed to link maternal BMI and child FA and MD. First, one possible explanation for the association of maternal BMI and differences in offspring white matter microstructure may relate to intrauterine programming and altered neurodevelopmental processes such as altered axonal development or myelination (35-37). Some of the hypotheses that have been proposed to underlie these associations are maternal inflammation (38) induced by obesity (39, 40), and the direct (41) and sensitizing (42) effects of maternal diet as shown in animal studies

Second, postnatal factors, such as maternal stress, parenting or breastfeeding, may explain the findings. Postnatal maternal obesity has been shown to predict poorer child psychosocial development including externalizing and internalizing behaviour, mediated by maternal stress (43). Breast milk in obese women has been shown to have pro-inflammatory properties and decreased levels of factors critical to neurodevelopment, such as fatty acids and carotenoids (44).

Third, the association of maternal BMI and offspring white matter microstructure could potentially be explained by a genetic or epigenetic vulnerability in the fetal period. Interestingly, maternal obesity has recently been related to fetal gene expression in a small study; approximately 700 genes that were differentially regulated were identified. These genes play a role in neurodevelopmental processes, inflammatory and immune signaling, glucose and lipid homeostasis, and oxidative stress (45).

Fourth, white matter development from childhood to early adulthood manifests as increasing FA with age (46). It could be possible that the association of maternal BMI and higher FA in offspring aged 10 and 26 years may suggest accelerated white matter development induced

by maternal obesity. This hypothesis is also supported by the finding of earlier menarche in female offspring of mothers with obesity (47).

It is essential to stress that many of the discussed mechanisms were only studied in animal models and thus we must be cautious interpreting the results of the current study. Furthermore, we cannot exclude the possibility of residual confounding, even though we controlled for various confounders. For example, we did not adjust for maternal diet during pregnancy, which has been shown to alter mesolimbic reward pathway in offspring brain (48) and alter brain cellular development (49, 50). Finally, it is also possible that our study suffers from insufficient power in demonstrating associations between maternal BMI and offspring white matter at 6 years in the PREOBE cohort.

Strengths and Limitations

The current study has several strengths. We used three different birth cohorts with neuroimaging data at different age ranges, and were able to use exactly the same processing pipeline. Nonetheless, our findings must be interpreted in the context of relevant limitations.

First, the cohorts only had a single neuroimaging measurement, so it was not possible to draw conclusions about the longitudinal associations of maternal prepregnancy BMI and white matter development. Future studies should focus on repeated neuroimaging in order to do so. Second, it could be possible that the associations observed reflect scanner differences, even though we used the same processing and analyses methodology. Further, each cohort had very different sample size which resulted in power differences and may have influenced the findings. In addition, inclusion and exclusion criteria were different across cohorts and could potentially

influence the findings. Finally, the three cohorts differed in terms of socioeconomic and lifestyle factors and this may also have influenced the findings.

Conclusions

Overall, we found that in three independent birth cohorts, maternal BMI was associated with higher FA and lower MD in multiple brain tracts in offspring aged 10 and 26 years, but not at 6 years. Future longitudinal studies should examine whether these associations persist in later ages and explore the causal nature of the findings.

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Supplementary information is available at International Journal of Obesity's website

Conflict of interest:

None

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Table 1: Scanner characteristics and acquisition parameters of diffusion weighted imaging in each cohort.

	PREOBE	Generation R	NFBC 1986
Scanner	3T Trio Siemens	3T GE MR750W	1.5T Siemens
Head coil (channels)	32	8	8
TR (ms)	3 300	12 500	9 000
TE (ms)	90	72	102
FOV (mm)	230 x 230	240 x 240	192 x 192
Matrix size	128 x 128	120 x 120	104 x 104
Number of slices	25	65	61
Voxel size (mm)	1.8 x 1.8 x 4	2 x 2 x 2	2.3 x 2.3 x 2.3
Directions	30	35	64
b-value	1 000	900	1 000
Acquisition time	5 min 18 s	7 min 40 s	8 min 25 s

Table note: TR: Repetition Time; TE: Echo Time; FOV: Field of View.

1 Table 2: Descriptive statistics of the study population

	PREOBE N = 116	Generation R N = 2466	NFBC 1986 N = 437
Maternal characteristics			
Age at study intake (mean, SD)	31.4 ± 4.2	30.9 ± 4.8	27.7 ± 5.4[1]
Country	Spain	The Netherlands	Finland
Date at intake	2008 - 2010	2002 - 2006	1985 - 1986
Maternal BMI	25.1 ± 4.4	24.4 ± 4.1	22.5 ± 3.8
Maternal weight categorization			
Underweight (BMI < 18.5)	0 (0 %)	45 (1.8 %)	36 (8.2 %)
Normal weight (18.5 ≤ BMI < 25)	71 (61.2 %)	1 576 (63.9 %)	326 (74.6 %)
Overweight (25 ≤ BMI < 30	26 (22.4 %)	604 (24.5 %)	50 (11.4 %)
Obese (BMI ≥ 30)	19 (16.4 %)	241 (9.8 %)	25 (5.7 %)
Educational level (%)			
Primary	16.4	6.7	34.7[2]
Secondary	38.8	39.7	49.0[3]
Higher	44.8	53.6	16.1
Ethnicity			
Spanish	100		
Dutch		58.4	
Non-Dutch Western		8.9	
Non-Dutch Non-Western		32.7	
Caucasian (Finns)			100
Alcohol use (%) [4]			
Never drank in pregnancy	73.2	38.9	85.5
Drank until pregnancy was known	6.3	13.9	
Continued to drink occasionally	15.2	36.9	

Continued to drink frequently	5.4	10.3	14.5
Smoking habits (%)			
Never smoked in pregnancy	67.6	73.9	54.5
Smoked until pregnancy was known	17.1	12.9	
Continued to smoke in pregnancy	15.3	13.2	45.5
Child Characteristics			
Sex (% boys)	50.9	49.4	42.1
Gestational age at birth (weeks)	39.4 ± 1.5	39.9 ± 1.8	39.7 ± 1.5
Birth weight (grams)	3330 ± 439	3435 ± 565	3570 ± 471
Age at MRI assessment (years)	6.02 ± 0.13	10.2 ± 0.6	26.4 ± 0.5
Height at assessment (cm)	119 ± 5.01	142 ± 6.5	170 ± 9.2
Age at height assessment (years)	6.02 ± 0.13	9.8 ± 0.4	26.4 ± 0.5

Table note: [1] Age at delivery; [2] Less than 8 years of primary school (10.6); 9-10 years primary school (24.1); [3] Vocational school or college 6-12 months (17.0), >1 year (32.0); [4] Frequent continued alcohol use is defined as '2 or more glasses of alcohol per week' in PREOBE, '1 or more glasses of alcohol per week in at least two trimesters' in Generation R, and '1.5 or more glasses of alcohol per week in at least two trimesters' in NFBC 1986.

Table 3. The association between maternal body mass index and fractional anisotropy of the white matter tracts.

White matter tracts of interest	Fractional anisotropy of the white matter tracts (FA)					
	PREOBE		**Generation R**		**NFBC 1986**	
Maternal body mass index - Zscore	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value
Brainstem tracts						
Medial lemniscus	-.004 (-.008 to .000)	.031	-.001 (-.002 to -.001)	.011#	.002 (.000 to .004)	.058
Middle cerebellar peduncle	-.001 (-.005 to .003)	.560	.022 (.000 to .003)	.028#	.001 (-.001 to .003)	.195
Callosal fibers						
Forceps minor	-.002 (-.010 to .005)	.550	-.002 (-.003 to -.000)	.021 #	.002 (-.001 to .005)	.236
Forceps major	-.006 (-.012 to .000)	.036	-.000 (-.002 to .002)	.992	.000 (-.003 to .003)	.956
Limbic system fibers						
Cingulate gyrus of the cingulum	-.004 (-.012 to .003)	.351	.002 (.000 to .004)	.019#	.002 (-.002 to .005)	.387
Parahippocampal part of the cingulum	.002 (-.003 to .008)	.371	.002 (.000 to .003)	.010#	.001 (-.002 to .004)	.640
Association fibers						
Superior longitudinal fasciculus	-.003 (-.008 to .002)	.199	.000 (-.001 to .001)	.652	.003 (.001 to .004)	.005 #
Inferior longitudinal fasciculus	-.002 (-.007 to .002)	.288	.000 (-.001 to .001)	.546	.001 (-.001 to .003)	.285
Inferior fronto-occipital fasciculus	.000 (-.005 to .005)	.885	.001 (.000 to .002)	.022#	.001 (-.001 to .003)	.161
Uncinate fasciculus	-.003 (-.008 to .002)	.254	.001 (.000 to .002)	.105	.003 (.000 to .005)	.023
Projection fiber						
Corticospinal tract	-.004 (-.009 to .002)	.199	.000 (-.001 to .001)	.667	.003 (.001 to .005)	.002 #
Acoustic radiation	.000 (-.005 to .004)	.883	.001 (.000 to .002)	.003#	.001 (-.001 to .003)	.497
Thalamic radiation	-.001 (-.004 to .002)	.566	.001 (.000 to .001)	.029#	.002 (.000 to .003))	.038

Table note: Linear regression analyses were used. B represents the association of maternal body mass index at intake and fractional anisotropy of white matter tracts in children. The adjusted regression models presented were adjusted for age and gender of the child, birth weight, maternal age, maternal smoking and drinking habits during pregnancy, maternal ethnicity, and educational level. Additional adjusting with child height at the time of imaging did not change the significance of results, except the results of the thalamic radiation in both Generation R and NFBC 1986. #These p-values survived the FDR correction for multiple testing. *Note*: No quadratic association between maternal body mass index and FA of the white matter tracts was observed.

Table 4. The association between maternal body mass index and mean diffusivity of the white matter tracts.

	Mean diffusivity of the white matter tracts (MD)					
	PREOBE		Generation R		NFBC 1986	
Maternal body mass index - Zscore	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value
Brainstem fibers						
Medial lemniscus	.000 (-.007 to .007)	.954	-.010 (-.031 to .011)	.358	-.005 -.008 to -.001)	.006 #
Middle cerebellar peduncle	-.003 (-.008 to .002)	.292	-.002 (-.005 to .002)	.369	-.002 (-.006 to .001)	.204
Callosal fibers						
Forceps minor	.001 (-.008 to .010)	.866	.000 (-.001 to .002)	.904	-.003 (-.006 to .001)	.117
Forceps major	.001 (-.012 to .014)	.889	-.001 (-.004 to .002)	.722	.005 (.000 to .011)	.042
Limbic system fibers						
Cingulate gyrus of the cingulum	.001 (-.004 to .006)	.716	-.001 (-.002 to .000)	.128	-.002 (-.006 to .003)	.447
Parahippocampal part of the cingulum	-.001 (-.010 to .008)	.791	-.003 (-.004 to -.001)	<.001#	-.001 (-.005 to .003)	.618
Association fibers						
Superior longitudinal fasciculus	.000 (-.004 to .005)	.937	-.001 (-.002 to -.000)	.042	-.002 (-.004 to .001)	.124
Inferior longitudinal fasciculus	.001 (-.005 to .006)	.816	-.001 (-.002 to .001)	.313	-.003 (-.006 to -.001)	.011 #
Inferior fronto-occipital fasciculus	.001 (-.004 to .005)	.788	-.001 (-.002 to .000)	.101	-.002 (-.004 to .000)	.074
Uncinate fasciculus	.000 (-.004 to .004)	.836	.000 (-.001 to .001)	.597	-.004 (-.006 to -.001)	.007 #
Projection fibers						
Corticospinal tract	.001 (-.004 to .005)	.742	-.001 (-.003 to .001)	.424	-.003 (-.006 to -.001)	.015 #
Acoustic radiation	-.002 (-.006 to .003)	.444	-.001 (-.002 to .000)	.085	-.001 (-.004 to .002)	.654
Thalamic radiation	.001 (-.003 to .005)	.571	-.001 (-.001 to .000)	.275	-.002 (-.004 to -.001))	.009 #

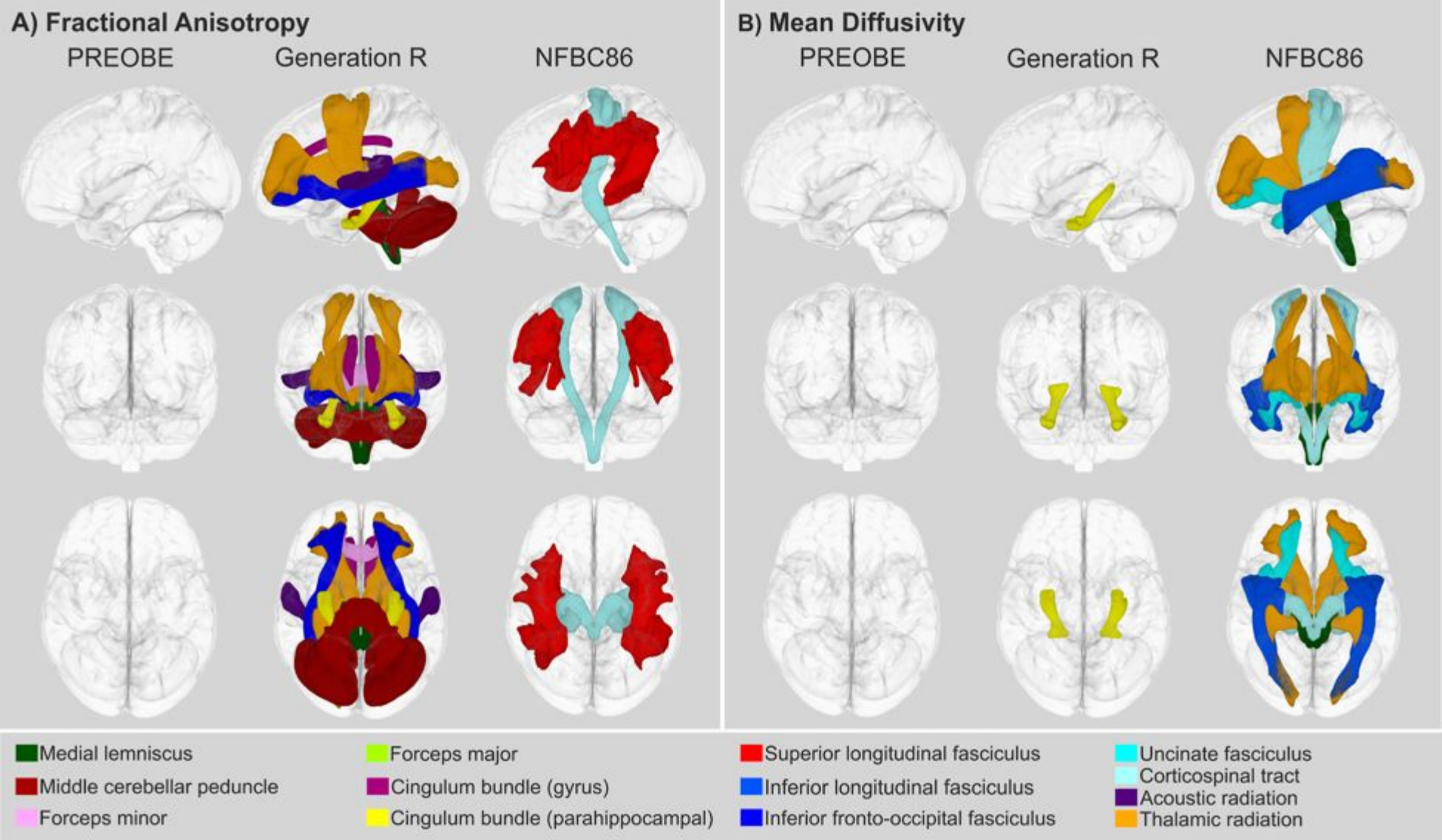
White matter tracts of interest

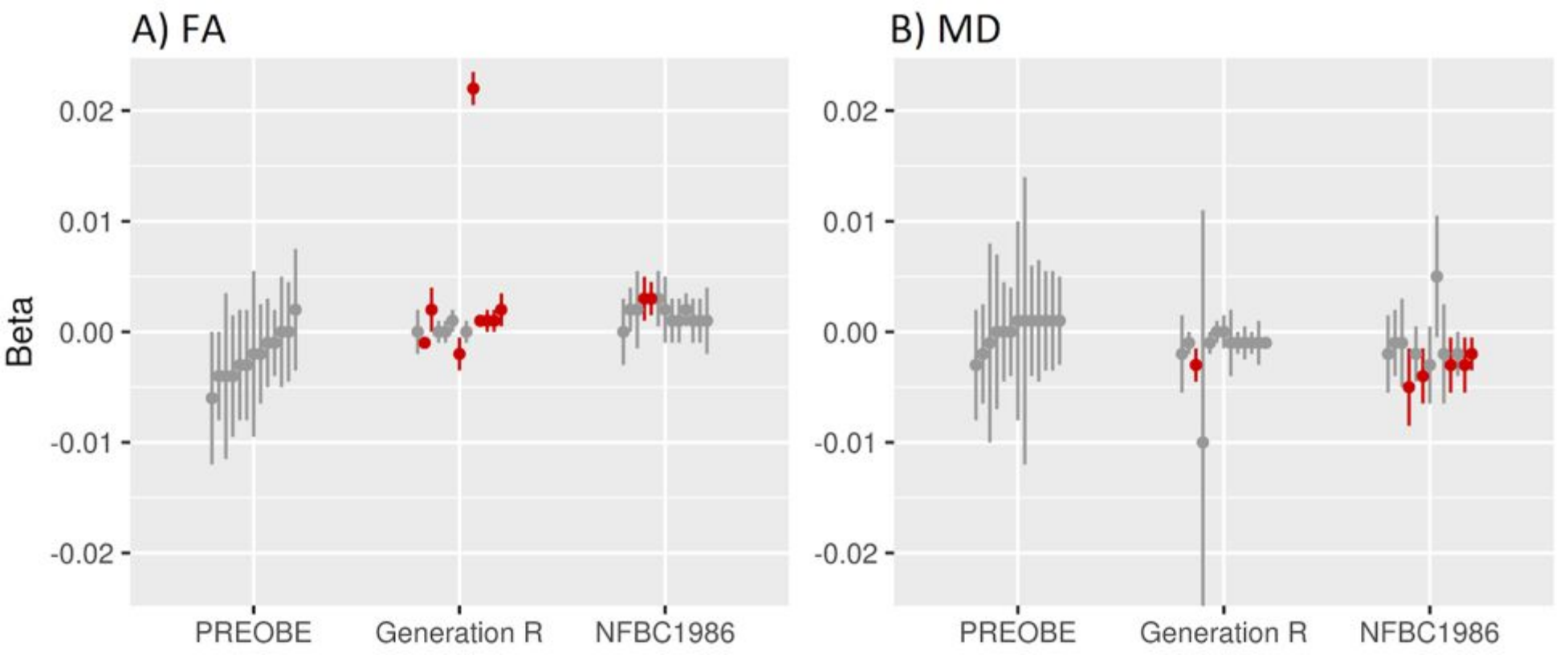
Table note: Linear regression analyses were used. B represents the association of maternal body mass index at intake and fractional anisotropy of white matter tracts in children. The adjusted regression models presented were adjusted for age and gender of the child, birth weight, maternal age, maternal smoking and drinking habits during pregnancy, maternal ethnicity, and educational level. Additional adjusting with child height at the time of imaging did not change the significance of results, except the results of the superior longitudinal fasciculus in the GR and the forceps major in the NFBC 1986 cohort. #These p-values (fully adjusted models) survived the FDR correction for multiple testing. *Note*: No quadratic association between maternal body mass index and MD of the white matter tracts was observed.

Figure Captions:

Figure 1. White matter tracts associated with maternal BMI. Only tracts surviving correction for multiple testing are presented. All models were adjusted for lifestyle and socioeconomic confounders including maternal age, smoking and drinking habits during pregnancy, maternal ethnicity, educational level, and birth weight, age and sex of the child. Panel A represents fractional anisotropy and panel B represents mean diffusivity. The images were created by averaging all individual maps by-tract in the NFBC 1986 sample and overlaying on the MNI152 brain template.

Figure 2. The relations of maternal BMI and microstructural parameters across cohorts. Beta estimates are mostly negative at 6 years and positive at 10 and 26 years in A) fractional anisotropy and *vice versa* in B) mean diffusivity. Estimates showing a significant association are coloured red. Beta estimates for the 13 tracts were ordered low-to-high in the PREOBE and in the same order in the other two samples.


A) Fractional Anisotropy
PREOBE
Generation R
NFBC86
B) Mean Diffusivity
PREOBE
Generation R
NFBC86
Medial lemniscus
Middle cerebellar peduncle
Forceps minor
Forceps major
Cingulum bundle (gyrus)
Cingulum bundle (parahippocampal)
Superior longitudinal fasciculus
Inferior longitudinal fasciculus
Inferior fronto-occipital fasciculus
Uncinate fasciculus
Corticospinal tract
Acoustic radiation
Thalamic radiation


A) FA
B) MD
Beta
0.02
0.01
0.00
-0.01
-0.02
PREOBE
Generation R
NFBC1986