Cardiovascular and Brain Health in Early Life

Carolina Costa Vicente Silva

Acknowledgements

The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMW), the Netherlands Organization for Scienti¬fic Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families.

The work presented in this thesis was conducted at the Generation R Study Group of the Erasmus Medical Center in Rotterdam.

Publication of this thesis was kindly supported by the Generation R Study Group and the Erasmus Medical Center, Rotterdam, the Netherlands.

ISBN: 978-94-6361-768-0

Cover design: Erwin Timmerman

Thesis layout and printing: Optima Grafische Communicatie (www.ogc.nl)

Cardiovascular and Brain Health in Early Life

Cardiovasculaire en brein gezondheid in het vroege leven

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr. A.L. Bredenoord

and in accordance with the decision of the Doctorate Board.

The public defense shall be held on
Wednesday 14 of December at 10:30 hours
by

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CONTENTS

MANUSCRIPTS BASED ON THIS THESIS

Chapter 2.1

Vehmeijer FOL, **Silva CCV**, Derks IPM, El Marroun H, Oei EHG, Felix JF, Jaddoe VWV, Santos S. Associations of maternal psychological distress during pregnancy with childhood general and organ fat measures. *Child Obes. 2019;15(5):313-322.*

Chapter 2.2

Silva CCV, Vehmeijer FOL, El Marroun H, Felix JF, Jaddoe VWV, Santos S. Maternal psychological distress during pregnancy and childhood cardio-metabolic risk factors. *Nutr Metab Cardiovasc Dis. 2019;29(6):572-9.*

Chapter 2.3

Monasso GS, **Silva CCV**, Santos S, Goncalvez R, Gaillard R, Felix JF, Jaddoe VWV. Infant weight growth patterns, childhood BMI, and arterial health at age 10 years. *Obesity (Silver Spring). 2022;30(3):770-8.*

Chapter 2.4

Silva CCV, Jaddoe VWV, Sol CM, El Marroun H, Martinez-Moral MP, Kannan K, Trasande L, Santos S. Phthalate and Bisphenol Urinary Concentrations, Body Fat Measures, and Cardiovascular Risk Factors in Dutch School-Age Children. *Obesity (Silver Spring). 2021;29(2):409-17.*

Chapter 3.1

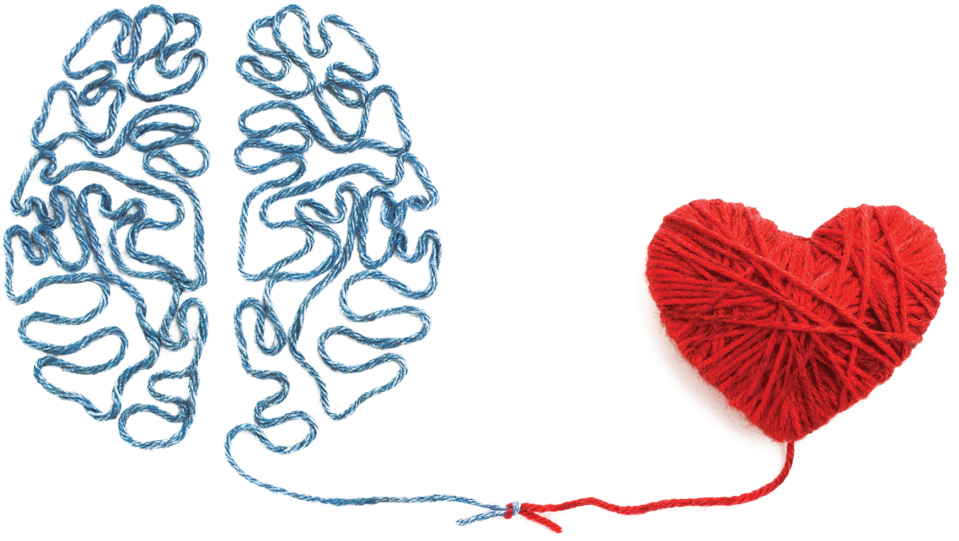
Silva CCV, Santos S, Muetzel RL, Vernooij M, van Rijn BB, Jaddoe VWV, El Marroun H. Maternal cardiovascular health in early pregnancy and childhood brain structure. *J Am Heart Assoc. 2022;11(19):e026133.*

Chapter 3.2

Silva CCV, El Marroun H, Sammallahti S, Vernooij M, Muetzel RL, Santos S, Jaddoe VWV. Patterns of fetal and infant growth and brain morphology at age 10 years. *JAMA Netw Open. 2021 Dec 1;4(12):e2138214.*

Chapter 3.3

Silva CCV, Jaddoe VWV, Muetzel RL, Santos S, El Marroun H. Body fat, cardiovascular risk factors and brain structure in school-age children. *Int J Obes (Lond). 2021;45(11):2425-31.*



Chapter 1

General introduction

Cardiovascular disease is a major public health problem and is the leading cause of mortality and morbidity in the general adult population worldwide, accounting for over 17.9 million deaths per year and representing approximately 32% of current mortality rates among men and women.[1] Next to cardiovascular disease, neurodegenerative disorders such as Alzheimer disease and other dementias are currently the seventh leading cause of death globally and one of the major causes of disability among the elderly.[2] Cardiovascular and brain health are closely linked and share some risk factors. Obesity, hypertension, diabetes and dyslipidemia are related to cardiovascular risk but also cerebrovascular injury and cognitive decline in older adults.[3, 4] Concurrent with the high prevalences among older age groups, the prevalence of obesity and related cardiovascular risk factors has also increased dramatically among children and adolescents.[5-8] The prevalence of neurodevelopmental disorders, such as intellectual disability, autism spectrum disorder and attention-deficit hyperactivity disorder is also high among children.[9, 10] An accumulating body of evidence suggests that obesity and adverse cardiovascular risk factors may be associated with deficits in neurocognitive and executive functions in childhood and adolescence.[11-14]

It has been proposed that obesity and cardiovascular diseases in childhood and adulthood may originate in early life, specifically fetal and early postnatal periods.[15-17] The Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that adverse exposures during critical periods in early-life lead to developmental adaptations that may have short and long term consequences for growth, body composition and cardiovascular health in later life.[18, 19] In addition to cardiovascular health, in the recent decades, the effects of early-life exposures on later neurodevelopmental outcomes have also gained attention. Studies among men and women who were born around the Dutch famine of 1944–1945 suggest that prenatal exposure to undernutrition irreversible affects the developing brain, resulting in altered brain structure and decreased cognitive function during adulthood.[20, 21]

Studying cardiovascular and brain health during childhood presents a unique opportunity to evaluate whether adverse early-life exposures may already trigger manifestations of poorer health before the development of clinically manifest diseases. Furthermore, this may help to develop future preventive strategies aimed at improving cardiovascular and brain health throughout the life course and in future generations. Therefore, the studies in this thesis are specifically focused on assessing the associations of early-life determinants, in particular psychological distress, growth, lifestyle and chemical exposures, with childhood cardiovascular and brain health (see overview in **Figure 1**).

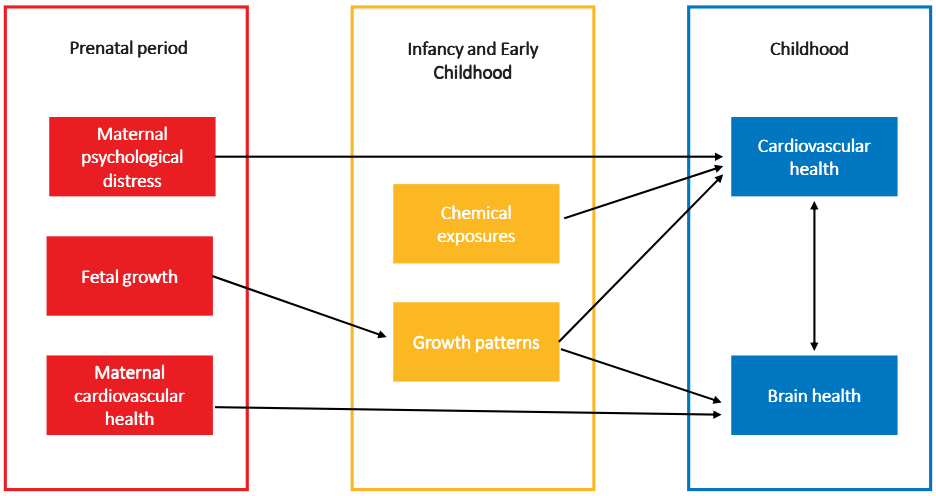


Figure 1. Overview of the assessed associations in this thesis.

CHILDHOOD CARDIOVASCULAR HEALTH

Obesity among children is increasing in prevalence worldwide. In 2020, an estimated 39 million children under the age of 5 years were overweight or obese.[22] Over the past decades, the prevalence of adverse cardiovascular risk factors, such as high blood pressure and altered lipid profile, has increased dramatically among children in concert with the global pandemic of obesity.[5-7] As adverse body fat and cardiovascular risk factors in childhood may contribute to the development of cardiovascular disease in adulthood, obtaining further insight into their determinants and underlying mechanisms is of importance for preventive strategies.

Early-life exposure to increased glucocorticoids levels might be an important biological mechanism linking adverse exposures in fetal life to cardiovascular outcomes.[23] Psychological distress during pregnancy increases maternal glucocorticoids concentrations leading to fetal developmental adaptations which may have persistent consequences for body composition and cardiovascular risk in later life.[24] Pregnancy might be a critical period for distress because of all physiological and psychological transformations.[25] Although some previous studies have observed an increased risk of obesity, high blood pressure and insulin resistance in children and adolescents exposed to prenatal psychological distress, results are not consistent.[26-31] Assessing the associations of maternal psychological distress during pregnancy with detailed childhood body fat and cardiovascular outcomes broadens the understanding of potential pathophysiological pathways starting already in early life and leading to adiposity and adverse cardiovascular profile later in life.

Infancy seems also to be a critical period for the development of an altered body composition and adverse cardiovascular profile.[32] Previous studies showed that higher infant peak

weight velocity and body mass index at adiposity peak are associated with an increased risk of childhood overweight, as well as with cardiovascular outcomes, such as higher blood pressure and lower left ventricular mass, already at school-age.[33-35] In addition to these cardiovascular risk factors, markers of arterial health, such as carotid intima-media thickness and distensibility, have also been used to assess cardiovascular risk from school-age to adulthood.[36-38] Insight into the patterns of weight growth in infancy and early childhood on arterial health at age 10 years is important from an etiologic perspective, as even subclinical differences in arterial health might predict cardiovascular risk and disease in adulthood.[39, 40]

Early-life exposure to environmental factors may also potentially influence body fat and cardiovascular outcomes in children. Endocrine disrupting chemicals, such as phthalates and bisphenols, are adverse environmental factors that may affect childhood health.[41, 42] Previous studies suggested that higher phthalate metabolites and bisphenol A concentrations were associated with adverse childhood body fat and cardiovascular profile.[43-47] Thus far, studies in children mostly used a cross-sectional design and did not show consistent results. Assessing the prospective associations of exposure to phthalate metabolites and bisphenols with body fat and cardiovascular profile in childhood will allow a better understanding of the influence of these chemicals on child's health.

Altogether, these studies suggest that fetal life, infancy and childhood may be critical periods for the development of an adverse body fat and cardiovascular profile from childhood onwards. Therefore, in this thesis, we assessed the associations of maternal psychological distress during pregnancy, infancy and childhood weight growth, and childhood chemicals exposures with childhood general and organ fat measures and cardiovascular risk factors.

CHILDHOOD BRAIN HEALTH

Human brain development is a complex ongoing process that starts soon after conception and extends throughout life. The embryonic period, from conception to 8 weeks of gestation, begins with the differentiation of the neural progenitor cells and ends with the formation of the neural tube, which is the first brain structure. The fetal period, from 9 weeks of gestation through the end of gestation, is characterized by extensive neuron production, migration, and differentiation, and the development of the mature pattern of gyral and sulcal folding. During this period, and especially during third trimester of gestation, there is a marked growth in brain size and a striking increase in volume of both the cerebrum and cerebellum.[48] Brain growth continues during the postnatal period throughout childhood and reaches approximately 90% of adult volume by age 6.[49] In addition, myelination is a largely postnatal process that peaks in early childhood.[50] Over the past twenty years, neuroimaging studies in children have been providing essential information about the living brain.[51] Assessing in vivo neuroimaging data broadens the understanding on the various facets of neurodevelopment,

allowing the identification of abnormal neurobiological processes. Brain morphological outcomes such as, for example, volumetric measures, can be quantified by a non-invasive magnetic resonance imaging (MRI).[52] In addition, microstructural properties of white matter integrity and architectural organization can be assessed by diffusion tensor imaging (DTI).[53]

The existing literature suggests that maternal obesity, hypertension and diabetes during pregnancy are associated with adverse neurodevelopmental and cognitive outcomes in the offspring.[54-57] A suboptimal intrauterine environment may lead to placental dysfunction, inflammation and changes in various metabolic processes, potentially altering fetal brain development, which may subsequently influence brain health in later life.[58, 59] Studies performed thus far have been mainly focused on maternal clinically manifest diseases and its association with childhood neurocognitive outcomes. Whether early pregnancy cardiovascular health also relates with childhood brain structure remains little investigated.

In addition to fetal life, infancy seems also to be a critical period for human brain development. Poor growth, both in utero and in infancy, seems to influence the risk of suboptimal neurodevelopment outcomes later in life.[60-63] Previous studies have mainly focused on infants born preterm or with low birth weight and have shown structural brain abnormalities and cognitive and behavioral dysfunctions during childhood and adolescence.[61-65] However, gestational age and weight at birth are merely the end point of fetal development and the starting point for infancy. Insight into the associations of fetal and infant growth patterns with brain structure will help to identify specific windows of vulnerability for brain development.

Furthermore, previous studies proposed that the associations of body fat measures and cardiovascular risk factors with brain outcomes may already be apparent years before the related adult diseases develop. For example, obesity and adverse cardiovascular profile have been associated with deficits in neurocognitive and executive functions among children and adolescents.[11-14] Thus far, studies have mainly focused on indirect measurements of adiposity such as BMI and remain limited on evaluating the association of cardiovascular risk factors with brain structure.

As brain structure and function may be influenced by early-life exposures it is important to obtain further insight into their determinants and underlying mechanisms. This may help to develop future preventive strategies aimed at improving brain health throughout the life course. Thus, in this thesis, we assessed the associations of patterns of fetal and infant growth and detailed maternal and childhood body fat and cardiovascular measures with brain structure, including volumetric measures and white matter microstructure, in 10-year-old children.

GENERAL AIMS OF THIS THESIS

The general aim of this thesis was to assess the associations of early-life determinants from fetal life to childhood with cardiovascular and brain health outcomes at age 10 years.

The specific aims of this thesis can be summarized as follows:

1. To assess the associations of maternal psychological stress during pregnancy, infancy and childhood weight growth, and childhood chemicals exposures with childhood cardiovascular outcomes
2. To assess the associations of fetal and infant growth patterns and detailed maternal and childhood body fat and cardiovascular measures with childhood brain developmental outcomes

General design

Studies in this thesis were embedded in the Generation R study. The Generation R study is an ongoing prospective population-based cohort study from early pregnancy until young adulthood in Rotterdam, the Netherlands. The Generation R study is designed to identify early environmental and genetic determinants of growth, development and health. Pregnant women residing in the study area with an expected delivery date between April 2002 and January 2006 were invited to participate. In total, 9,778 mothers were enrolled in the study, of whom 8880 (91%) were included during pregnancy. Enrolment was aimed at early pregnancy, but was possible until birth. Data collection was planned in early-, mid-, and late-pregnancy (<18, 18-25 and ≥25 weeks of gestation, respectively) and included parental physical examinations, blood and urine samples, fetal ultrasound examinations, and self-administered questionnaires. Detailed follow-up assessments of mothers and children occurred in the preschool period (0-4 years), early childhood (6 years), mid-childhood (10 years), and early adolescence (13 years).[66] Currently, participants are being evaluated at 17 years old.

The studies presented in this thesis used data from pregnancy, birth, infancy and the follow-up visits at 6 and 10 years of age. Information on pre-pregnancy weight, lifestyle factors and symptoms of psychological distress was obtained from questionnaires during pregnancy.[66, 67] General body fat was measured with a Dual-energy X-ray absorptiometry (DXA) scanner at 6 and 10 years and abdominal and organ fat measures were obtained from magnetic resonance imaging (MRI) at 10 years.[66] Biological samples were taken including urine samples to measure phthalate and bisphenol concentrations at 6 years, and blood samples to measure cardiovascular risk factors at 10 years.[68, 69] In addition, children participated in a neuroimaging session that included high-resolution structural and diffusion tensor MRI sequences at 10 years.[52]

Outline of the thesis

The specific aims of this thesis are addressed in the studies presented in **Chapter 2** and **Chapter 3**. **Chapter 2** of this thesis focuses on childhood cardiovascular health. In **Chapter 2.1**, we examined the associations of maternal psychological distress during pregnancy with childhood general and organ fat measures. The associations of maternal psychological distress during pregnancy with childhood cardio-metabolic risk factors are described in **Chapter 2.2**.

Chapter 2.3 focuses on the influence of infant growth patterns and childhood body mass index on early markers of atherosclerosis in school-age children. In **Chapter 2.4**, we studied the associations of phthalate and bisphenol concentrations in children with childhood body fat measures and cardiovascular risk factors.

Chapter 3 of this thesis focuses on childhood brain health and its link with childhood cardiovascular health. In **Chapter 3.1**, we assessed whether maternal body fat measures and cardiovascular risk factors in early pregnancy are associated with childhood brain structure. Subsequently, we studied the associations of patterns of fetal and infant growth with brain morphology at age 10 years (**Chapter 3.2**). Lastly, in **Chapter 3.3** we examined whether childhood body fat measures and cardiovascular risk factors are associated with brain structure in school-age children.

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Chapter 2

Childhood cardiovascular health

Chapter 2.1

Maternal psychological distress during pregnancy and childhood general and organ fat measures

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Adapted from: *Child Obes. 2019 Jul;15(5):313-322.*

ABSTRACT

Background: Psychological distress during pregnancy may influence offspring adiposity. No studies assessed the associations with organ fat measures. We examined the associations of maternal psychological distress, depression and anxiety during pregnancy with child general and organ fat measures.

Methods: In 4,161 mother-offspring pairs, psychological distress was self-reported in pregnancy. We obtained general fat measures including BMI and fat mass index by dual-energy X-ray absorptiometry and organ fat measures (in a subsample of 2,447 children) including subcutaneous, visceral, and pericardial fat indices and liver fat fraction by Magnetic Resonance Imaging at 10 years. Linear and logistic regression models were used.

Results: Children of mothers with psychological distress had higher fat mass index (difference 0.14 (95% Confidence Interval (CI) 0.04, 0.24) standard deviation scores (SDS)) and higher risk of obesity (Odds Ratio (OR) 1.73 (95% CI 1.09, 2.74). Maternal anxiety was associated with higher BMI (difference 0.16 (95% CI 0.05, 0.26) SDS), fat mass index (difference 0.19 (95% CI 0.10, 0.28) SDS) and higher risks of overweight and obesity (OR 1.36 (95% CI 1.03, 1.81), 1.78 (95% CI 1.13, 2.81)). Maternal anxiety was associated with higher subcutaneous and visceral fat indices and liver fat fraction (differences 0.16 (95% CI 0.03, 0.29), 0.15 (95% CI 0.01, 0.29), 0.16 (95% CI 0.02, 0.29) SDS). No associations were observed for maternal depression.

Conclusions: Psychological distress and anxiety, but not depression, during pregnancy were associated with higher child general and organ fat measures. A healthy mental state during pregnancy may be important for preventing child adiposity.

INTRODUCTION

Psychological distress is common during pregnancy, affecting 10-20% of pregnant women.[1-4] Psychological distress is mostly defined as perceived stress, depressive symptoms, anxiety or experiencing an adverse life event.[2, 5] Maternal psychological distress during pregnancy is associated with several adverse fetal outcomes such as intra-uterine growth retardation and low birth weight.[6, 7] Psychological distress during pregnancy may lead to developmental adaptations of the fetus, which may have persistent consequences for body composition in later life.[8, 9] One of the most described potentially involved mechanisms includes fetal hypothalamic-pituitary-adrenal (HPA) axis dysregulation in response to increased maternal stress hormones like cortisol.[10, 11] Previous studies showed that an altered fetal HPA axis is associated with an increased risk of adiposity later in life.[12, 13] Although various studies have observed an increased risk of obesity in children exposed to prenatal psychological distress, results are not consistent.[14-20] BMI is easy to obtain, but does not give insight on the body fat distribution. The Framingham Heart Study and the Jackson Heart Study have reported that excess visceral, pericardial and liver fat are related to various cardio-metabolic abnormalities, independently of BMI.[21-24] To date, no studies assessed the association between maternal psychological distress and childhood organ fat measures.

We hypothesized that psychological distress during pregnancy is associated with childhood general and organ fat measures. We examined, in a population-based prospective cohort study among 4,161 mothers and their children, the associations of maternal overall psychological distress, depression and anxiety during pregnancy with offspring BMI, fat mass index measured by dual-energy X-ray absorptiometry (DXA) and subcutaneous fat index, visceral fat index, pericardial fat index and liver fat fraction measured by Magnetic Resonance Imaging (MRI) at 10 years.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy until young adulthood onwards in Rotterdam, the Netherlands.[25] The study was approved by the local Medical Ethics Committee of Erasmus MC (MEC 198.782/2001/31). Pregnant women were enrolled between 2002 and 2006. Of all the eligible children in the study area, 61% participated at birth in the study. Written informed consent was obtained for all mothers and children. In total, 8,879 mothers were enrolled during their pregnancy.[26] We excluded pregnancies not leading to singleton live births (N = 246). Of 8,633 mothers and their singleton children, information about psychological distress during pregnancy was available in 6,548 mothers. For 2,387 children, no information on any measurement

of adiposity at 10 years was available. Thus, the population for analysis consisted of 4,161 mothers and their children (**Figure S1** in Supplementary Materials).

Psychological distress during pregnancy

Information on maternal psychological distress was obtained through questionnaires that were mailed to participants and completed at approximately 20 weeks of gestation. The Brief Symptom Inventory (BSI) is a validated self-report questionnaire consisting of 53 items.[27] These items describe multidimensional psychopathology symptoms that mothers may have experienced in the preceding 7 days. The items are divided in 9 subscales (including anxiety, depression, hostility, phobic anxiety, interpersonal sensitivity, obsessive-compulsiveness, paranoid ideation, psychoticism and somatization).

As an indicator of overall psychological distress, we used the Global Severity Index (GSI), that is a total score of the 9 subscales. Additionally, we used the depression and anxiety subscales separately. We chose these 2 subscales, because they are widely used as valid proxies for psychological distress during pregnancy. [2, 5] Moreover, relating depression and anxiety, separately, with childhood adiposity could uncover different associations, as hypothesized by previous studies. [28]

The items were rated on a 5-point unidimensional scale ranging from '0' (not at all) to '4' (extremely) indicating to what extent the symptom was experienced. A total score was provided for each symptom scale by summing the item scores and dividing the results by the number of reported symptoms. Higher scores represented an increased occurrence of overall psychological symptoms. Then, women were categorized according to the presence of 'clinically' significant psychological symptoms (into "yes" or "no" categories) by using the following cut-offs derived from a psychiatric outpatient sample of Dutch women: 0.71 for the overall psychological symptoms scale; 0.80 for the depression scale and 0.71 for the anxiety scale.[29, 30] In the group of women with symptoms of overall psychological distress (>0.71), depression (>0.80) or anxiety (>0.71), we considered moderate or severe stress, depression or anxiety if below or above the 85th percentile of our study population, respectively.

Measures of adiposity at 10 years

As previously described, children around the age of 10 years were invited to visit our research center at the Erasmus MC-Sophia Children's Hospital to participate in hands-on measurements.[25] We calculated BMI (kg/m^2) at this age from height and weight, both measured without shoes and heavy clothing. We calculated sex- and age- adjusted standard deviation scores (SDS) of childhood BMI based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[31] Child BMI categories (underweight, normal weight, overweight and obesity) were calculated using the International Obesity Task Force cut-offs.[32, 33] We measured total body fat mass using a DXA scanner (iDXA, GE140 Lunar, 2008,

Madison, WI, USA, enCORE software v.12.6), according to standard procedures.[34] Previous studies showed that DXA can accurately measure body fat.[35]

Measures of organ fat at 10 years were obtained from MRI scans.[25] MRI has been considered the gold standard for the measurement of intra-abdominal and organ fat deposition because it is an accurate and reproducible technique.[36] Children were scanned using a 3.0 Tesla MRI (Discovery MR 750w, GE Healthcare, Milwaukee, WI, USA) for body fat imaging using standard imaging and positioning protocols, while performing expiration breath-hold maneuvres of maximum 11 seconds duration. They wore light clothing without metal objects while undergoing the body scan.[37] Pericardial fat imaging in short axis orientation was performed using an ECG triggered black-blood prepared thin slice single shot fast spin echo acquisition (BB SSFSE) with multi-breath-hold approach. An axial 3-point Dixon acquisition for fat and water separation (IDEAL IQ) was used for liver fat imaging. This technique also enables the generation of liver fat fraction images.[38] An axial abdominal scan from lower liver to pelvis and a coronal scan centered at the head of the femurs were performed with a 2-point DIXON acquisition (LavaFlex).

The obtained fat scans were subsequently analyzed by the Precision Image Analysis company (PIA, Kirkland, Washington, United States), using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually.[36] Pericardial fat included both epicardial- and paracardial fat directly attached to the pericardium, ranging from the apex to the left ventricular outflow tract. Total subcutaneous and visceral fat volumes were generated by summing the volumes of the liver, abdominal and if necessary the femoral fat-only scans, encompassing the fat volume ranging from the dome of the liver to the superior part of the femoral head. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined by taking four samples of at least 4 cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat fraction estimation. To create measures independent of height, we estimated the optimal adjustment by log-log regression analyses and subsequently we divided total and subcutaneous fat mass by $height^4$ (fat mass index and subcutaneous fat index) and visceral and pericardial fat mass by $height^3$ (visceral and pericardial fat indices) (More details given in **Supplemental Methods**).[39, 40]

Covariates

We obtained information on maternal age, pre-pregnancy BMI, ethnicity (European vs non-European), educational level, marital status, smoking habits, alcohol consumption during pregnancy, and folic acid supplement use, by questionnaire at enrollment. Smoking habits, higher alcohol consumption and inadequate folic acid supplement use may be related to childhood adiposity and may be more frequent in pregnant women with psychological distress as these women are more likely to adopt unhealthy behaviors.[41-44] Information on child

sex was available from medical records. Information on the average child television watching time was obtained by questionnaires at the age of 10 years filled out by the mother.

Statistical analysis

First, we performed descriptive statistics to gain better understanding on the differences between women with and without psychological distress and between participants and non-participants. Second, we used linear regression models to assess the associations of maternal overall psychological distress, depression and anxiety with offspring adiposity measures at 10 years (BMI, fat mass index, subcutaneous, visceral and pericardial fat indices and liver fat fraction). Third, we used multinomial logistic regression models to assess the associations of maternal overall psychological distress, depression and anxiety with the risk of childhood underweight, overweight or obesity. Fourth, to explore whether the associations differ by severity of stress, we assessed the associations of moderate and severe levels of maternal overall psychological distress, depression and anxiety with childhood adiposity measures at 10 years.

We used a basic model including child sex and age at outcome measurements, and a confounder model, which additionally included all aforementioned covariates. We identified potential covariates based on the graphical criteria for confounding or due to the relation with the outcomes by visualizing a directed acyclic graph (DAG) and included the covariates in the models that changed the effect estimates >10%. [45, 46] **Figure S2** shows a DAG depicting the covariates included in the models. We log-transformed the non-normally distributed childhood DXA and MRI adiposity measures. We constructed SDS [(observed value - mean)/ SD] of the sample distribution for DXA and MRI outcomes to enable comparisons of effect sizes. No statistical interactions of maternal psychological distress with maternal ethnicity and child sex were observed in the associations with all childhood adiposity measures. We hypothesized that psychological distress is associated with higher child general and organ fat measures. Since we tested a single hypothesis with several exposures and outcomes, correction for multiple testing seems unnecessary.[47] To enable interpretation of statistical significance level, we presented p-values < 0.05 and p-values < 0.01. In order to maintain statistical power and reduce bias related to missing data on covariates we performed multiple imputation according to Markov Chain Monte Carlo method[48]. The percentage of missing data on covariates ranged from 0 to 21%. Psychological distress and adiposity measures were used as predictor variables only and were not imputed. Covariates were imputed and used as predictor variables. Five imputed datasets were created and pooled results are presented. No substantial differences in descriptive statistics were found between the original and imputed datasets. All statistical analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (IBM SPSS Inc, Chicago, IL, USA).

RESULTS

Participant characteristics

In total, 8.6%, 8.6% and 9.6% of all pregnant women experienced psychological distress, depression and anxiety, respectively. **Table 1** shows the participant characteristics. Women with psychological distress were more often younger, non-European, lower educated, without partner, with higher pre-pregnancy BMI and were more likely to be smokers compared to women without psychological distress. Mothers of children with follow-up data available were slightly older, more often European, higher educated and reported less psychological distress compared to mothers of children without follow-up data available (**Table S1** in Supplementary Materials).

Maternal psychological distress and childhood general fat measures

Table 2 shows that, in the basic models, maternal overall psychological distress, depression and anxiety during pregnancy were associated with higher childhood BMI and fat mass index (p-values<0.05). After adjustment for potential confounders, maternal overall psychological distress was associated with higher childhood fat mass index (difference 0.14 (95% Confidence Interval (CI) 0.04,0.24)), and maternal anxiety was associated with higher childhood BMI and fat mass index (differences 0.16 (95% CI 0.05,0.26) SDS, 0.19 (95% CI 0.10, 0.28) SDS, respectively). The associations for maternal depression attenuated and were no longer significant. When comparing the models with and without each specific covariate, we observed that inclusion of maternal educational level and child television watching time caused the largest change in effect estimates (>45% and >28%, respectively).

Figure 1 shows that in both basic and confounder models, none of the maternal psychological stress scales were associated with the risk of childhood underweight. On the contrary, in the basic models, all maternal stress scales were associated with an increased risk of childhood overweight and obesity (p-values<0.05). After adjustment for confounders, overall psychological distress remained associated with an increased risk of childhood obesity (Odds Ratio (OR) 1.73 (95% CI 1.09, 2.74)), but not overweight. Maternal anxiety remained associated with increased risks of childhood overweight (OR 1.36 (95% CI 1.03, 1.81)) and obesity (OR 1.78 (95% CI 1.13, 2.81)). No significant associations were observed for depression in the confounder model.

When we explored whether the associations differed by severity of maternal psychological distress, depression or anxiety, we observed stronger effect estimates for BMI and fat mass index when mothers had severe rather than moderate stress, depression or anxiety, but the confidence intervals overlapped and thus the differences were not statistically significant (**Figure S3** in Supplementary Materials).

Table 1. Characteristics of mothers and their children (N= 4,161)[1]

	Total group (N= 4,161)	No psychological distress (N= 3,802)	Psychological distress (N= 359)
Maternal characteristics			
Age at intake, mean (SD), years	30.9 (4.8)	31.2 (4.6)	28.1 (5.8)
Ethnicity, N (%)			
European	2,818 (68.3)	2,712 (71.8)	106 (30.4)
Non-European	1,309 (31.7)	1,066 (28.2)	243 (69.6)
Education, N (%)			
Primary school	256 (6.4)	200 (5.4)	56 (17.2)
Secondary school	1,660 (41.2)	1,459 (39.4)	201 (61.7)
High education	2,115 (52.5)	2,046 (55.2)	69 (21.2)
Marital status, N (%)			
Married/living together	3,565 (89.1)	3,324 (90.8)	241 (71.3)
No partner	434 (10.9)	337 (9.2)	97 (28.7)
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.1, 34.2)	22.5 (18.1, 34.0)	23.1 (17.9, 36.1)
Alcohol consumption during pregnancy, N (%)			
Yes	2,254 (59.7)	2,114 (61.1)	140 (44.7)
No	1,519 (40.3)	1,346 (38.9)	173 (55.3)
Smoking during pregnancy, N(%)			
Yes	920 (24.1)	784 (22.4)	136 (42.2)
No	2,897 (75.9)	2,711 (77.6)	186 (57.8)
Folic acid supplement use, N (%)			
No	657 (20.0)	547 (18.0)	110 (45.3)
Start during first 10 weeks	1,047 (31.9)	963 (31.7)	84 (34.6)
Preconceptional use	1,581 (48.1)	1,532 (50.4)	49 (20.2)
Child characteristics			
Sex, N (%)			
Boys	2,034 (48.9)	1,839 (48.4)	195 (54.3)
Girls	2,127 (51.1)	1,963 (51.6)	164 (45.7)
Age at visit, mean (SD), years	9.8 (0.3)	9.8 (0.3)	9.8 (0.4)
Height, mean (SD), cm	141.6 (6.6)	141.7 (6.6)	140.6 (7.0)
BMI, median (95% range), kg/m^2	16.9 (14.0, 24.6)	16.9 (14.0, 24.0)	17.8 (13.9, 27.6)
BMI categories, N (%)			
Underweight	292 (7.0)	271 (7.2)	21 (5.9)
Normal weight	3,143 (75.8)	2,915 (76.9)	228 (63.9)
Overweight	573 (13.8)	500 (13.2)	73 (20.4)
Obesity	139 (3.4)	104 (2.7)	35 (9.8)
Total fat mass, median (95% range), g	8,450 (4,466, 21,648)	8,355 (4,466, 20,930)	9,806 (4,408, 27,184)
Subcutaneous fat mass, median (95% range), g	1,305 (599, 5,319)	1,294 (599, 4,974)	1,480 (577, 7,184)
Visceral fat mass, median (95% range), g	367 (164, 969)	366 (164, 950)	370 (161, 1178)

Table 1. Characteristics of mothers and their children (N= 4,161)[1] (*continued*)

	Total group (N= 4,161)	No psychological distress (N= 3,802)	Psychological distress (N= 359)
Pericardial fat mass, median (95% range), g	11 (5, 23)	11 (5, 23)	11 (4, 22)
Liver fat fraction, median (95% range), %	2.0 (1.2, 5.2)	2.0 (1.2, 4.9)	2.1 (1.2, 10.6)
Television watching time at 10 years, N (%)			
< 2 hours/day	2,352 (69.8)	2,228 (71.0)	124 (52.8)
≥ 2 hours/day	1,019 (30.2)	908 (29.0)	111 (47.2)

1 Values are observed data and represent means (standard deviation), medians (95% range) or numbers of participants (valid %)

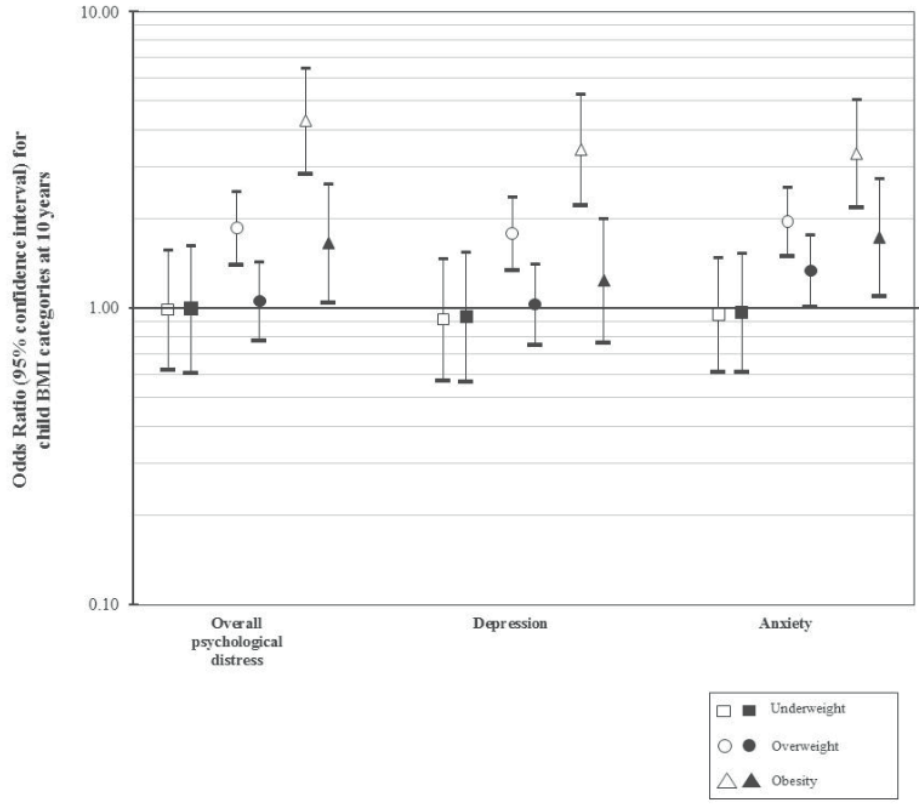

Figure 1. Associations of maternal psychological distress, depression and anxiety scales with childhood BMI clinical categories at 10 years (N=4,147)[1]

[1]Values are odds ratios (95% Confidence Intervals) on a logarithmic scale and represent the risk of childhood underweight, overweight and obesity at 10 years for maternal overall psychological distress, depression and anxiety compared to no psychological distress, depression or anxiety. Basic models (□) include child´s sex and age. The confounder models (■) are additionally adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid supplement use and child television watching time.

Table 2. Associations of maternal psychological distress, depression and anxiety scales with childhood general fat measures at 10 years (N= 4,147)[1]

Maternal psychological distress scales	Measures of general fat at 10 years in SDS			
	Body Mass Index (n=4,147)		Fat Mass Index (n=4,097)	
	Basic Model	Confounder Model	Basic Model	Confounder Model
Overall distress				
No Stress	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.40 (0.29,0.51)**	0.10 (-0.02,0.21)	0.49 (0.38,0.59)**	0.14 (0.04,0.24)**
Depression				
No Depression	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.36 (0.25,0.47)**	0.06 (-0.05,0.17)	0.39 (0.28,0.49)**	0.06 (-0.04,0.16)
Anxiety				
No Anxiety	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.37 (0.26,0.48)**	0.16 (0.05,0.26)**	0.43 (0.33,0.53)**	0.19 (0.10,0.28)**

[1]Values are linear regression coefficients (95% confidence interval) and reflect the change in SDS childhood BMI and fat mass index for stress, depression and anxiety, compared to the reference group. Basic models include child´s sex and age (except for sex- and age-adjusted body mass index SDS). Confounder models are additionally adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid use and child TV watching time. *p < 0.05. ** p < 0.01.

Maternal psychological distress and childhood organ fat measures

Table 3 shows that, in the basic models, maternal overall psychological distress, depression and anxiety during pregnancy were associated with higher childhood subcutaneous fat index and liver fat fraction (p-values<0.05). Maternal anxiety was also associated with higher visceral fat index in the offspring (p-values<0.05). We did not observe associations of maternal psychological distress, depression and anxiety with childhood pericardial fat index. After adjustment for potential confounders, only maternal anxiety remained associated with higher childhood subcutaneous fat index (difference 0.16 (95% CI 0.03, 0.29) SDS), visceral fat index (difference 0.15 (95% CI 0.01, 0.29) SDS) and liver fat fraction (difference 0.16 (95% CI 0.02, 0.29) SDS). When comparing the models with and without each specific covariate, we observed that inclusion of maternal educational level and BMI before pregnancy caused the largest change in effect estimates (>60% and >30%, respectively). We observed a tendency towards stronger associations with organ fat measures when mothers had severe rather than moderate stress, depression or anxiety but the differences were not statistically significant (**Figure S4** in Supplementary Materials).

Table 3. Associations of maternal psychological distress, depression and anxiety scales with childhood organ fat measures at 10 years (N = 2,447)[1]

Maternal psychological distress scales	Measures of organ fat at 10 years in SDS							
	Subcutaneous fat index (n=2,141)		Visceral fat index (n=2,141)		Pericardial fat index (n=2,210)		Liver fat fraction (n=2,410)	
Overall distress	**Basic Model**	**Confounder Model**	**Basic Model**	**Confounder Model**	**Basic Model**	**Confounder Model**	**Basic Model**	**Confounder Model**
No Stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.35 (0.20,0.50)**	0.03 (-0.12,0.18)	0.15 (-0.00,0.31)	0.04 (-0.11,0.20)	-0.05 (-0.21,0.11)	-0.08 (-0.24,0.08)	0.23 (0.09,0.37)**	0.09 (-0.06,0.24)
Depression								
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.30 (0.15,0.45)**	0.02 (-0.13,0.17)	0.13 (-0.02,0.29)	0.06 (-0.10,0.22)	-0.06 (-0.22,0.10)	-0.07 (-0.24,0.10)	0.22 (0.07,0.36)**	0.10 (-0.05,0.25)
Anxiety								
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.39 (0.25,0.52)**	0.16 (0.03,0.29)*	0.24 (0.10,0.38)**	0.15 (0.01,0.29)*	0.06 (-0.09,0.20)	0.04 (-0.11,0.18)	0.26 (0.13,0.40)**	0.16 (0.02,0.29)*

[1]Values are linear regression coefficients (95% confidence interval) and reflect the change in SDS childhood subcutaneous, visceral, pericardial fat indices and liver fat fraction for overall psychological distress, depression and anxiety, compared to no psychological distress, depression or anxiety. Basic models include child´s sex and age. The confounder models are additionally adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid use and child TV watching time. *p < 0.05. ** p < 0.01.

DISCUSSION

In this population-based prospective cohort study, we observed that maternal psychological distress and anxiety during pregnancy were associated with higher general fat measures and an increased risk of overweight and obesity in the offspring. Maternal anxiety was also associated with higher subcutaneous, visceral and liver fat at 10 years. No associations were observed for maternal depression with childhood general and organ fat measures.

Interpretation of main findings

Psychological distress has been reported by 10-20% of women during pregnancy.[2, 4, 49] We observed a slightly lower but still considerable prevalence of overall psychological distress (8.6%), depression (8.6%) or anxiety (9.6%) in pregnancy. Previous studies examining the association of maternal psychological distress during pregnancy with childhood adiposity mainly focused on BMI. Most studies reported that maternal distress during pregnancy is associated with an increased risk of childhood overweight and obesity.[14, 18, 50, 51] For example, in a study among 65212 mother-child pairs, 10-13-year old children exposed to prenatal stress, defined by being born to mothers who were bereaved by death of a close family member, had an increased risk of overweight.[14] On the other hand, the same study found no significant association between prenatal maternal stress and the risk of overweight in children younger

than 10 years.[14] Likewise, 5-year-old children exposed to maternal psychological distress during pregnancy because of job strain did not have an increased BMI or fat mass index.[52] Also, we previously reported absence of an association between prenatal stress of the mother and offspring BMI in children aged 3 months to 3 years.[15] In the present study, we observed that overall psychological distress and anxiety during pregnancy were associated with an increased risk of childhood obesity and higher child fat mass index, anxiety was additionally associated with higher childhood BMI and an increased risk of child overweight at 10 years. Thus, maternal psychological distress during pregnancy seems not to influence fat mass development in early childhood, but the effects seem to become more apparent at older offspring ages.

Large studies such as the Framingham Heart study and the Jackson Heart Study have reported the important effect of excess ectopic fat deposition on an adverse cardio-metabolic risk profile in adults. [21-24] Addressing the influence of maternal psychological distress during pregnancy on organ fat measures in addition to general fat measures gives a more complete understanding of the health risks in children. To our knowledge, the association between maternal distress during pregnancy and childhood organ fat measures has not been studied yet. In the present study, we observed that maternal anxiety during pregnancy was associated with higher subcutaneous fat index, visceral fat index and liver fat fraction in their 10-year old children. Remarkably, overall psychological distress and depression were not associated with childhood organ fat measures. Thus, maternal anxiety but not overall psychological distress and depression during pregnancy seems to influence organ fat development and ultimately the cardio-metabolic health in the offspring.

The influence of maternal psychological distress during pregnancy on general and organ fat measures might be dependent on the levels of stress. Only one previous study investigated a dose-response relation between antenatal depression and childhood adiposity and found no evidence for such a relation.[17] Although we observed a tendency towards stronger associations with childhood adiposity measures when mothers reported severe rather than moderate stress, depression or anxiety, the differences between severe and moderate stress effect estimates were not statistically significant. Only a few women reported severe levels of stress in this study, compromising the statistical power and complicating the detection of significant associations. Thus, the findings may suggest that increasing levels of psychological distress are associated with increased childhood adiposity measures, which emphasizes the need to reduce the severity of psychological distress in pregnant women.

There are various pathways through which maternal psychological distress during pregnancy may affect offspring adiposity.[11] Fetal programming of body composition, obesity and metabolic function could be influenced by maternal psychological distress during pregnancy.[53] The most frequently described mechanism includes fetal HPA axis dysregulation due to maternal stress hormones during pregnancy, which subsequently affects fetal and child growth and adiposity.[10, 11, 13] Other mechanisms involving the autonomic nervous system,

maternal microbiome, (epi)genetics and inflammatory factors may also play an important (mediating) role.[11] However, from the current observational data, no conclusions can be drawn on the causality of the observed associations. Unmeasured lifestyle-related characteristics might also partly explain the associations. Psychological distress during pregnancy is related to an adverse maternal health behavior such as an unhealthy diet.[54] If antenatal psychological distress continues to be present after birth, this risk behavior may affect the body fat development of the child through, for example, parenting and dietary habits.[55] The consistent associations observed in this study for anxiety with general and organ fat measures and the absence of associations for depression suggest that the mechanisms might be dependent on the specific psychiatric symptom experienced or might be more pronounced with anxiety rather than with depressive symptoms. A tendency for maternal anxiety during pregnancy, and not depression, being associated with offspring outcomes was already observed in the same population as the current study but with fetal growth outcomes.[28]

Our study shows that maternal psychological distress and anxiety during pregnancy are associated with higher child general and organ fat measures, which have important adverse cardio-metabolic health consequences. Further studies, using paternal psychological distress as a negative control exposure, are needed to obtain insight into the causality of the observed associations and the underlying biological mechanisms. If these associations are shown to be causal, promoting a healthy mental state during pregnancy is needed for a healthy fat mass development and ultimately to improve cardio-metabolic health of the offspring.

Strengths and limitations

Strengths of this study were the prospective data collection from pregnancy onwards, the large sample size and data available on detailed childhood adiposity measures including organ fat measures assessed by MRI. This study also has limitations. Of all women included during pregnancy with a singleton live-born child, 76% responded to the questionnaire. Only 64% of the children had information on at least one measurement of adiposity at 10 years. Non-response could lead to selection bias if the associations of maternal psychological distress with childhood adiposity measures differ between mothers and children included and excluded in the analyses. As shown in the non-response analyses, mothers of children with and without follow-up data were different regarding the socioeconomic background and frequency of psychological distress. We believe selection bias has little influence on our findings since we adjusted for covariates associated with loss to follow-up such as maternal sociodemographic and lifestyle related factors.[56] Inverse probability weighting techniques were not further applied since previous studies showed no additional effect on the estimates.[56] Another limitation is that we relied on self-report measurements of maternal psychological distress, which may have resulted in underreporting of psychological symptoms and subsequently in an underestimation of observed effects. Maternal psychological distress was measured at only one time point during pregnancy, on average at 20 weeks, and

refers to the preceding 7 days. Therefore we do not know whether maternal psychological distress symptoms varied in intensity or were persistent throughout pregnancy. There are no clinical cut-offs to differentiate between moderate and severe levels of psychological distress. We used the 85th percentile, which has been used as cut-off to distinguish risk levels in other fields of research.[57] Future studies should assess the optimal cut-off to classify mothers according to the severity of stress symptoms. The BSI showed good accuracy to identify clinical depression and anxiety in a healthy and outpatient psychiatric population but has not been validated to date in pregnant women.[28] Finally, although we adjusted the analyses for many sociodemographic, lifestyle, and other variables known to influence the associations, residual confounding, for example by maternal nutritional intake, income, chronic conditions and physical activity, might still be present in our study.

CONCLUSION

Our results suggest that maternal overall psychological distress during pregnancy is associated with higher general fat measures and anxiety with higher general and organ fat measures in children aged 10 years. Our findings emphasize the importance of promoting a healthy mental state during pregnancy as it may have long-term consequences on child health.

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SUPPLEMENTARY MATERIAL

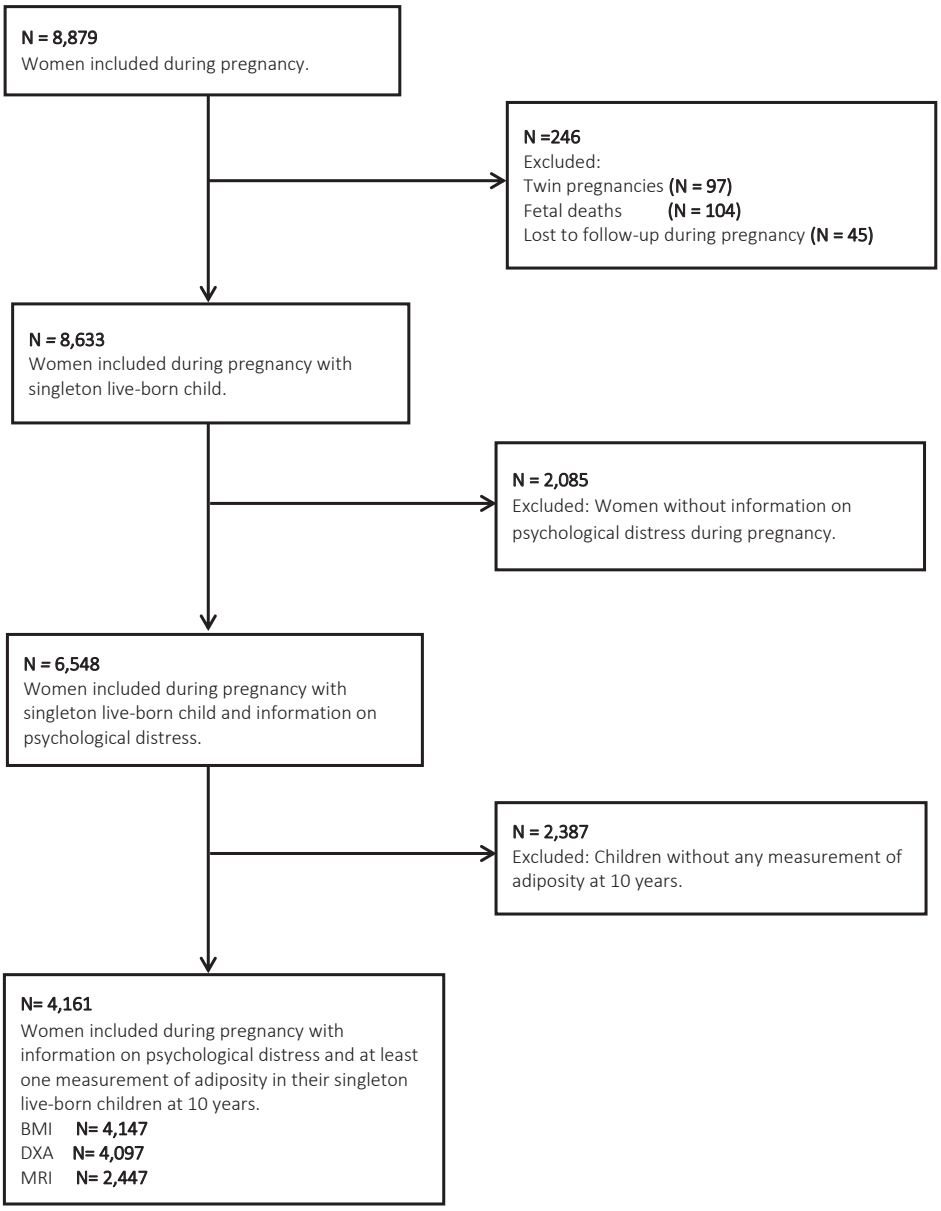

Figure S1. Flowchart of study population
Abbreviations: BMI = Body Mass Index, DXA = Dual-energy X-ray absorptiometry MRI = Magnetic Resonance Imaging

SUPPLEMENTAL METHODS: LOG-LOG REGRESSION ANALYSES

We estimated the optimal adjustment by log-log regression analyses in order to create measures of child adiposity independent of height at 10 years.[1] Total fat mass, subcutaneous fat mass, visceral fat mass and pericardial fat mass and height were log-transformed, using natural logs. Log-adiposity measures were regressed on log-height. To calculate an index uncorrelated with height, we took the regression slope as the power by which height should be raised. Thus, we divided total fat mass by height^4, subcutaneous fat mass by height^4, visceral fat mass by height^3, and pericardial fat mass by height^3.

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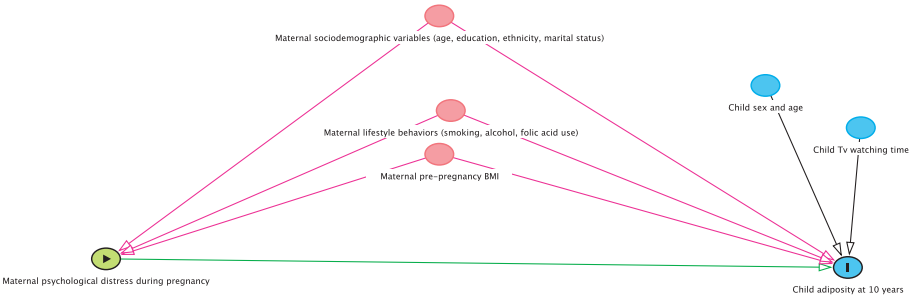

Figure S2. Directed acyclic graph (DAG) for the relationship between maternal psychological distress during pregnancy and child adiposity measures at 10 years depicting the covariates included in the models

Table S1. Comparison of maternal and child characteristics between mothers and children with and without follow-up data available (N = 6,548)[1]

	With follow-up (N= 4,161)	Without follow-up (N= 2,387)
Maternal characteristics		
Age at intake, mean (SD), years	30.9 (4.8)	28.4 (5.4)
Ethnicity, N (%)		
European	2,818 (68.3)	1,136 (49.9)
Non-European	1,309 (31.7)	1,141 (50.1)
Education, N (%)		
Primary school	256 (6.4)	360 (14.5)
Secondary school	1,660 (41.2)	1,164 (52.7)
High education	2,115 (52.5)	725 (32.8)
Marital status, N (%)		
Married/living together	3,565 (89.1)	1,823 (82.2)
No partner	434 (10.9)	395 (17.8)
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.1, 34.2)	22.6 (17.7, 35.4)
Alcohol consumption during pregnancy, N (%)		
Yes	2,254 (59.7)	968 (45.3)
No	1,519 (40.3)	1,167 (54.7)
Smoking during pregnancy, N (%)		
Yes	920 (24.1)	694 (31.9)
No	2,897 (75.9)	1,481 (68.1)
Folic acid supplement use, N (%)		
No	657 (20.0)	641 (35.3)
Start during first 10 weeks	1,047 (31.9)	581 (32.0)
Preconceptional use	1,581 (48.1)	593 (32.7)
Overall psychological distress, N (%)		
Yes	359 (8.6)	351 (14.7)
No	3,802 (92.4)	2,036 (85.3)
Child characteristics		
Sex, N (%)		
Boys	2,034 (48.9)	1,226 (51.4)
Girls	2,127 (51.1)	1,161 (48.6)

[1] Values are observed data and represent means (standard deviation), medians (95% range) or numbers of participants (valid %).

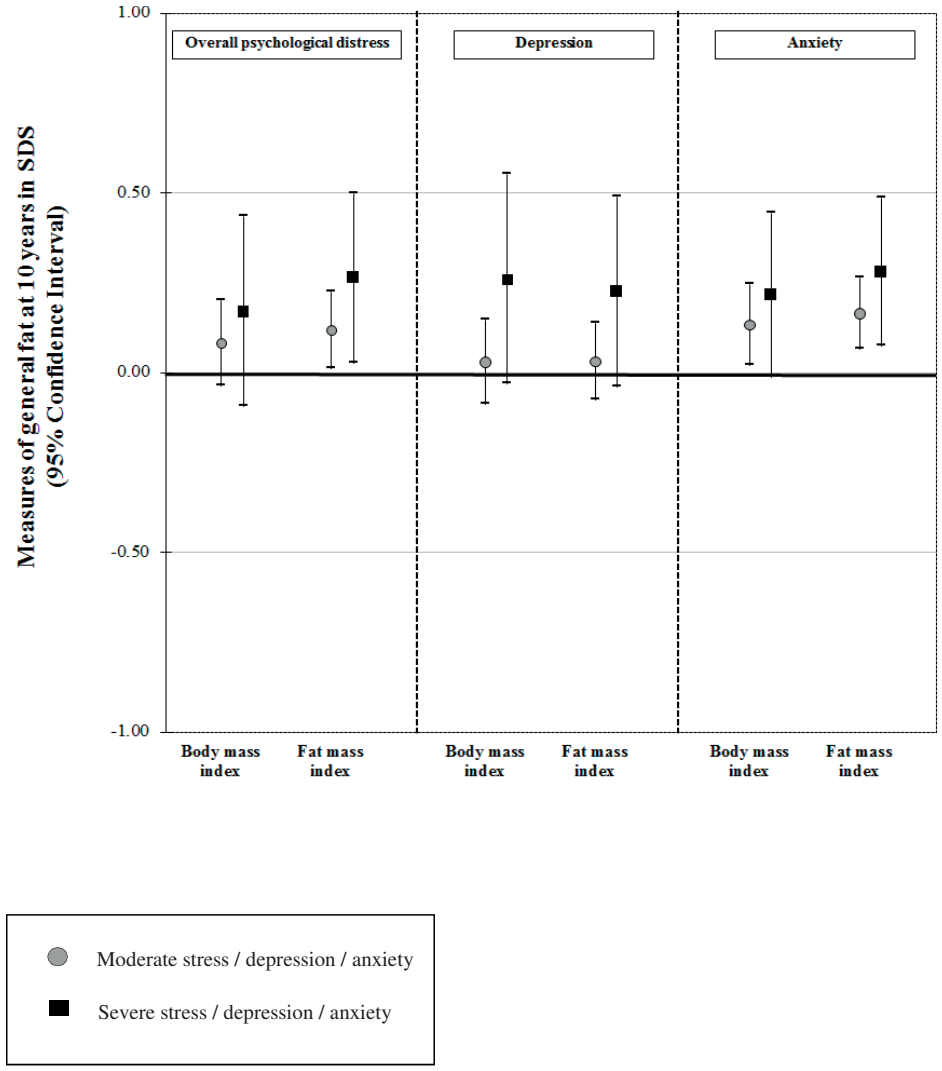

Figure S3. Associations of moderate and severe maternal psychological distress, depression and anxiety with childhood general fat measures at 10 years (N = 4,147)[1]

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in SDS in childhood BMI and fat mass index for severe and moderate maternal psychological stress, depression or anxiety as compared to no psychological stress, depression or anxiety. Only the confounder models are shown which are adjusted for child sex and age, maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid supplement use and child television watching time.

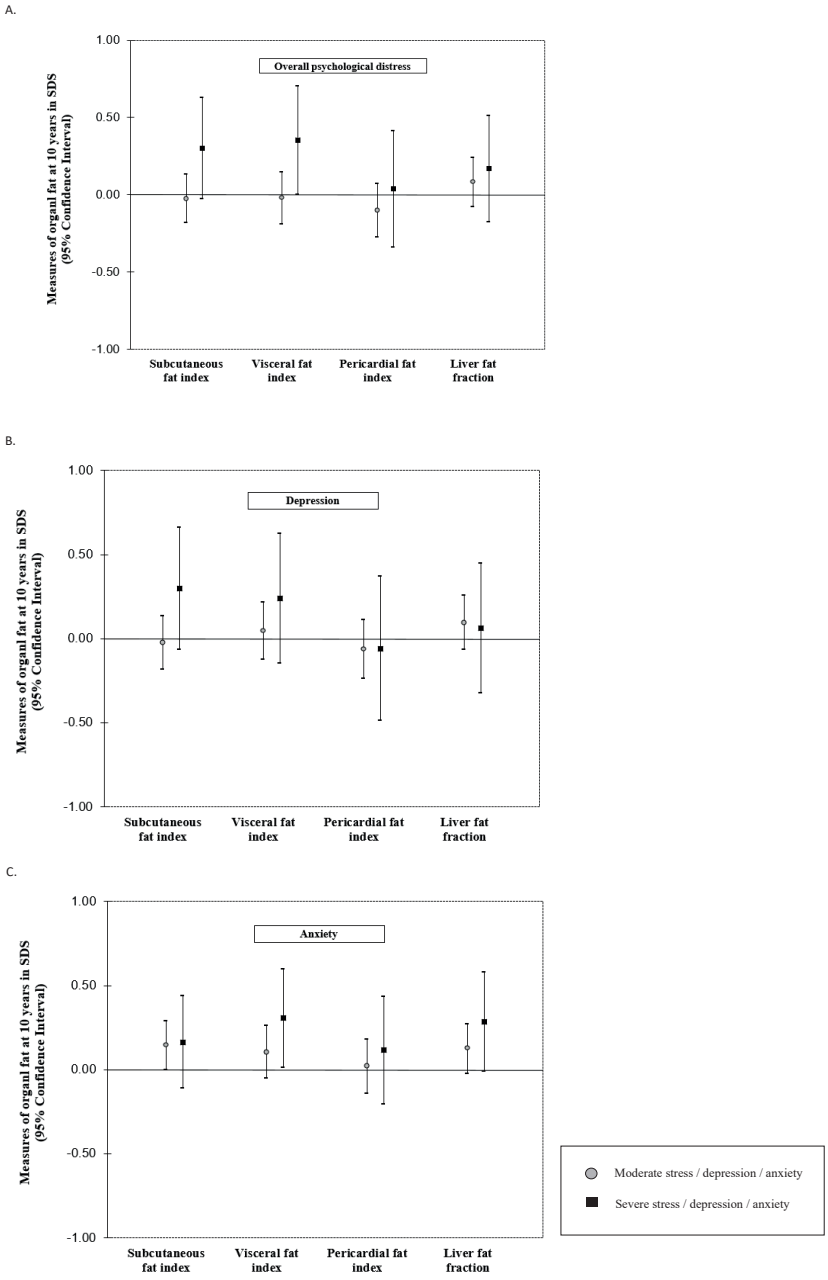

Figure S4. Associations of moderate and severe maternal psychological distress, depression and anxiety with childhood organ fat measures at 10 years (N = 2,447)[1]

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in subcutaneous, visceral, pericardial fat indices and liver fat fraction for severe and moderate maternal psychological stress (A), depression (B) or anxiety (C) as compared to no psychological stress, depression or anxiety. Only the confounder models are shown which are adjusted for child sex and age, maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid supplement use and child television watching time.

Chapter 2.2

Maternal psychological distress during pregnancy and childhood cardiovascular risk factors

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Adapted from: *Nutr Metab Cardiovasc Dis. 2019 Jun;29(6):572-579*

ABSTRACT

Background and Aims: Previous studies suggest that psychological distress during pregnancy may lead to fetal developmental adaptations, which programme cardio-metabolic disease of the offspring. We examined the associations of maternal overall psychological distress, depression and anxiety during pregnancy with cardio-metabolic risk factors in 10-year-old children and explore potential sex-specific differences.

Methods and results: In a population-based prospective cohort study among 4,088 mothers and their children, information about overall psychological distress, including depression and anxiety was obtained through the Brief Symptom Inventory during pregnancy. We measured child blood pressure and heart rate and insulin, glucose, serum lipids and C-reactive protein blood concentrations at 10 years. Analyses were performed in the total group and in boys and girls separately. Psychological distress during pregnancy was associated with higher childhood heart rate among boys only (differences 0.34 (95% Confidence Interval (CI) 0.18, 0.50) standard deviation scores (SDS), 0.22 (95% CI 0.06, 0.38) SDS, 0.33 (95% CI 0.19, 0.48) SDS, for overall psychological distress, depression and anxiety, respectively). Maternal anxiety during pregnancy was associated with higher childhood triglycerides among girls (difference 0.35 (95% CI 0.17, 0.53) SDS). Maternal psychological distress was not associated with childhood blood pressure, cholesterol, insulin, glucose and C-reactive protein concentrations.

Conclusions: Maternal psychological distress may influence their offspring heart rate and triglycerides concentrations. Further studies are needed to replicate these findings and assess the long-term cardio-metabolic consequences of maternal psychological distress.

INTRODUCTION

Pregnancy is a period of great physiological and psychological transformations.[1] Psychological distress has been reported by 10-20% of women during pregnancy.[2] Maternal psychological distress may cause a suboptimal intrauterine environment leading to long-term consequences on growth and health of the offspring.[3, 4] More specifically, intrauterine stress exposure may affect offspring cardio-metabolic development via dysregulation of the hypothalamic-pituitary-adrenal axis, increase of inflammatory responses and changes in the balance of the autonomic nervous system.[5-7] In addition, growing evidence suggested sex-specific differences in fetal programming in response to stress, which may result in sex-specific risks for later diseases.[8, 9] We have previously reported that maternal psychological distress during pregnancy was not associated with offspring infant heart rate and early-childhood blood pressure.[7, 10] Other studies reported inconsistent associations of distress during pregnancy with blood pressure and insulin resistance in children and adolescents.[11-14] To date, no studies have focused on the associations of maternal psychological distress during pregnancy with childhood lipids profile or inflammatory markers. Insight into the associations of maternal distress during pregnancy with childhood cardio-metabolic risk factors may help to develop future preventive strategies.

We examined, in a population-based prospective cohort study among 4,088 mothers and their children, the associations of maternal overall psychological distress, depression and anxiety during pregnancy with blood pressure, heart rate, lipids profile, glucose metabolism, and C-reactive protein concentrations in 10-year-old children. We explored whether the associations with cardio-metabolic risk factors differ for boys and girls.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands. The study was approved by the local Medical Ethics Committee of Erasmus MC (MEC 198.782/2001/31). Pregnant women were enrolled between 2002 and 2006. Written informed consent was obtained for all participants. In total, 8,879 mothers were enrolled during prenatal period.[15] We excluded pregnancies not leading to singleton live births (N = 246). Information about psychological distress during pregnancy was available in 6,548 of 8,633 mothers with singleton children. For 2,460 children, no information on any measurement of cardio-metabolic risk factors at 10 years was available. Thus, 4,088 mothers and children had information on psychological distress during pregnancy and at least one measurement of cardio-metabolic risk factors at 10 years. The specific population for analysis for each outcome is shown in the flowchart. (**Figure S1** in Supplementary Materials).

Psychological distress during pregnancy

Information on maternal psychological distress was obtained through the Brief Symptom Inventory (BSI) that was mailed to participants and returned at around 20 weeks of gestation. The BSI is a validated self-report questionnaire with 53 items, describing the psychopathologic problems and complaints that mothers may have experienced in the preceding 7 days.[16] These items include a broad spectrum of psychological symptoms, divided in 9 dimensions (anxiety, depression, hostility, phobic anxiety, interpersonal sensitivity, obsessive-compulsiveness, paranoid ideation, psychoticism, somatization). We used the overall psychological distress scale (Global Severity Index) and 2 symptom scales (depression and anxiety) to define psychological distress. We chose these subscales because depression and anxiety are widely used as indicators of psychological distress during pregnancy.[1] To indicate the extent of the symptoms, the items were rated on a 5-point unidimensional scale ranging from '0' (not at all) to '4' (extremely). A total score was provided for each symptom scale by summing the item scores and dividing the results by the number of reported symptoms. Then, the symptoms were dichotomized (into "yes" or "no" categories) by using the following cutoffs derived from a psychiatric outpatient sample of Dutch women: 0.71 for overall psychological symptoms scale; 0.80 for depression scale and 0.71 for anxiety scale.[17, 18]

Cardio-metabolic risk factors at 10 years

As previously described, children around the age of 10 years were invited to visit our research center at Erasmus MC-Sophia Children's Hospital.[19] Blood pressure and heart rate were measured at the right brachial artery four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ).[20] We calculated the mean value for systolic and diastolic blood pressure and heart rate using the last three measurements of each participant. Non-fasting blood samples were collected to determine serum concentrations of glucose, insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides. Glucose, total cholesterol, HDL-cholesterol and triglycerides concentrations were measured using the c702 module on the Cobas 8000 analyzer. Insulin was measured with electrochemiluminescence immunoassay (ECLIA) on the E411 module (Roche, Almere, the Netherlands).[21] Low-density lipoprotein (LDL)–cholesterol was calculated according to the Friedewald formula.[22]

Covariates

We obtained information on maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, smoking habits and alcohol consumption during pregnancy, and folic acid supplement use, by questionnaire. Information on maternal selective serotonin reuptake inhibitors (SSRIs) use in pregnancy was obtained by questionnaires and prescription records from pharmacies.[23] Information on child sex, gestational age at birth and birth weight were available from medical records. We calculated body mass index (kg/m^2) at 10 years from height and weight, both measured without shoes and heavy clothing.

Statistical analysis

We compared subject characteristics between women with and without psychological distress using Pearson's chi-square tests, independent sample t-tests and Mann-Whitney tests. Similar statistical tests were performed to compare characteristics between participants and non-participants. We used linear and logistic regression models to assess the associations of maternal overall psychological distress, depression and anxiety with childhood cardio-metabolic risk factors. We included covariates in the models if they were associated with maternal psychological distress and childhood cardio-metabolic risk factors in our study and if they changed the effect estimates substantially (>10%) for at least one outcome. Thus, all models were adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking, folic acid and selective serotonin reuptake inhibitors use during pregnancy. Child body mass index at 10 years might be in the causal pathway of the associations of maternal overall psychological distress with childhood cardio-metabolic risk factors. We assessed whether these associations were independent of child body mass index, by additionally adjusting our models for this covariate. The distributions of insulin and triglycerides were skewed and natural logged transformed. Since C-reactive protein was not normally distributed and the log-transformation did not yield an acceptable distribution, we categorized C-reactive protein concentrations into <3 mg/l (normal levels) or ≥3 mg/l (high levels) in line with previous studies.[24] To enable comparison of effect sizes of different outcome measures, we constructed standard deviation scores (SDS) ((observed value – mean) / SD). Analyses were performed for the total group and for boys and girls, separately. We found statistically significant sex interactions for the associations of maternal psychological distress with child heart rate and diastolic blood pressure. We did not observe statistical interactions for maternal ethnicity, child's gestational age at birth, birth weight and body mass index at 10 years. To enable interpretation of statistical significance level, we presented p-values<0.05 and p-values<0.01. Missing data in covariates (ranging from 0 to 21%) were multiple-imputed using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. All statistical analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS IBM, Chicago, IL, USA).

RESULTS

Subject characteristics

Participants characteristics are presented in **Table 1**. Of all pregnant women, 8.5%, 8.6% and 9.5% experienced overall psychological distress, depression and anxiety, respectively. Women with psychological distress during pregnancy were more often younger, non-European, lower educated, without partner and were more likely to be smokers compared to women without psychological distress (p-values<0.05). Non-response analyses showed that mothers of children

with follow-up data available were slightly older, more often European, higher educated and reported less clinical psychological distress during pregnancy compared to mothers of children without follow-up data available (p-values<0.05) (**Table S1** in Supplementary Materials).

Table 1. Characteristics of mothers and their children[1]

	Total group (N= 4,088)	Overall psychological distress (N= 352)	No overall psychological distress (N= 3,736)	P-value[2]
Maternal characteristics				
Age at intake, mean (SD), years	30.9 (4.8)	28.1 (5.8)	31.2 (4.6)	< 0.001
Ethnicity, N(%)				< 0.001
European	2,767 (68.2)	104 (30.4)	2,663 (71.7)	
Non-European	1,288 (31.8)	238 (69.6)	1,050 (28.3)	
Education, N(%)				< 0.001
Primary school	255 (6.4)	56 (17.6)	199 (5.5)	
Secondary school	1,628 (41.1)	195 (61.1)	1,433 (39.4)	
High education	2,076 (52.4)	68 (21.3)	2,008 (55.2)	
Marital status, N(%)				< 0.001
Married/living together	3,502 (89.2)	236 (71.3)	3,266 (90.8)	
No partner	425 (10.8)	95 (28.7)	330 (9.2)	
Pre-pregnancy body mass index, median (95% range) kg/m^2	22.6 (18.1, 34.3)	23.2 (17.9, 36.1)	22.5 (18.1, 34.0)	< 0.05
Alcohol consumption, N (%)				< 0.001
Yes	2,219 (59.9)	137 (44.6)	2,082 (61.3)	
No	1,486 (40.1)	170 (55.4)	1,316 (38.7)	
Smoking, N (%)				< 0.001
Yes	901 (24.0)	132 (41.9)	769 (22.4)	
No	2,847 (76.0)	183 (58.1)	2,664 (77.6)	
Folic acid supplement use, N (%)				< 0.001
No	650 (20.1)	108 (44.8)	542 (18.2)	
Start during first 10 weeks	1,030 (31.9)	84 (34.9)	946 (31.7)	
Preconceptional use	1,546 (47.9)	49 (20.3)	1,497 (50.2)	
Exposed to SSRIs, N (%)				< 0.001
Yes	43 (1.1)	12 (3.7)	31 (0.9)	
No	3,823 (98.9)	314 (96.3)	3,509 (99.1)	
Child characteristics				
Sex, N (%)				0.06
Boys	1,987 (48.6)	188 (53.4)	1,799 (48.2)	
Girls	2,101 (51.4)	164 (46.6)	1,937 (51.8)	
Gestational age at birth, N (%)				< 0.05
Preterm (< 37 weeks)	178 (4.4)	23 (6.5)	155 (4.1)	
Term (≥ 37 weeks)	3,910 (95.6)	329 (93.5)	3,581 (95.9)	
Birth weight[3], N (%)				< 0.05

Table 1. Characteristics of mothers and their children[1] (*continued*)

	Total group (N= 4,088)	Overall psychological distress (N= 352)	No overall psychological distress (N= 3,736)	P-value[2]
Small for gestational age	405 (9.9)	48 (13.7)	357 (9.6)	
Appropriate for gestational age	3,270 (80.1)	277 (78.9)	2,993 (80.2)	
Large for gestational age	409 (10.0)	26 (7.4)	383 (10.3)	
Age at visit, mean (SD), years	9.8 (0.3)	9.8 (0.4)	9.8 (0.3)	< 0.05
Body mass index, median (95% range), kg/m^2	16.9 (14.0, 24.5)	17.8 (13.9, 27.7)	16.9 (14.0, 24.0)	< 0.001
Systolic blood pressure, mean (SD), mmHg	103.1 (8.0)	104.8 (8.9)	102.9 (7.9)	< 0.001
Diastolic blood pressure, mean (SD), mmHg	58.5 (6.4)	59.7 (7.0)	58.4 (6.4)	< 0.001
Heart rate, mean (SD), beats/minute	73.5 (10.0)	76.7 (10.7)	73.2 (9.9)	< 0.001
Insulin, median (95% range), pmol/L	172.9 (35.2, 642.6)	206.8 (40.7, 824.6)	170.2 (34.6, 637.5)	< 0.05
Glucose, mean (SD), mmol/L	5.2 (0.9)	5.2 (0.9)	5.2 (0.9)	0.77
Total-cholesterol, mean (SD),mmol/L	4.3 (0.7)	4.3 (0.7)	4.3 (0.7)	0.53
HDL-cholesterol, mean (SD), mmol/L	1.5 (0.3)	1.4 (0.3)	1.5 (0.3)	< 0.05
LDL-cholesterol, mean (SD), mmol/L	2.3 (0.6)	2.3 (0.6)	2.3 (0.6)	0.96
Triglycerides, median (95% range), mmol/L	1.0 (0.4, 2.6)	1.0 (0.4, 3.0)	1.0 (0.4, 2.5)	0.32
C-reactive protein, median (95% range), mg/L	0.3 (0.3, 5.2)	0.3 (0.3, 12.4)	0.3 (0.3, 4.9)	< 0.001

[1] Values are means (standard deviation), medians (95% range) or numbers of subjects (valid %).

[2] P-values for differences in subject characteristics between groups were calculated performing independent sample t-tests for normally distributed continuous variables, Mann-Whitney test for not normally distributed continuous variables and chi-square tests for categorical variables.

[3] Sex- and gestational age-adjusted birth weight SDS were created based on a North-European reference chart. Small and large size for gestational age at birth were defined as sex- and gestational age-adjusted birth weight below the 10th percentile and above the 90th percentile, respectively.

Maternal psychological distress and childhood blood pressure and heart rate

In the unadjusted models, maternal overall psychological distress, depression and anxiety during pregnancy were associated with higher childhood blood pressure in the total group and among boys (p-values<0.05). Maternal overall distress and anxiety were also associated with higher childhood systolic and diastolic blood pressure, respectively among girls (p-values<0.05). All maternal psychological distress scales were associated with higher childhood heart rate among boys and girls (p-values<0.05) (**Table S2** in Supplementary Materials). After adjustment for potential confounders, no associations were observed between maternal overall psychological distress, depression and anxiety and childhood blood pressure in boys and girls. All maternal psychological distress scales remained associated with higher childhood heart rate only among boys (differences 0.34 (95% Confidence Interval (CI) 0.18,0.50) SDS, 0.22 (95% CI 0.06,0.38) SDS, 0.33 (95% CI 0.19, 0.48) SDS for overall distress, depression and anxiety, respectively) (**Table 2**). After additional adjustment for child body mass index, similar associations of maternal psychological distress scales with childhood blood pressure and heart rate were observed (**Table S3** in Supplementary Materials).

Table 2. Associations of maternal psychological distress scales with childhood blood pressure and heart rate at 10 years for the total group and stratified for boys and girls[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Systolic blood pressure			Diastolic blood pressure			Heart rate		
	Total group (n=4,011)	**Boys** (n=1,945)	**Girls** (n=2,066)	**Total group** (n=4,011)	**Boys** (n=1,946)	**Girls** (n=2,065)	**Total group** (n=3,954)	**Boys** (n=1,918)	**Girls** (n=2,036)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.09 (-0.03, 0.20)	0.12 (-0.03, 0.28)	0.06 (-0.11, 0.23)	0.07 (-0.04, 0.19)	0.11 (-0.05, 0.27)	0.03 (-0.14, 0.20)	0.23 (0.12, 0.35)**	0.34 (0.18, 0.50)**	0.14 (-0.03, 0.31)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.01 (-0.10, 0.13)	0.02 (-0.14, 0.18)	0.01 (-0.16, 0.18)	0.05 (-0.07, 0.16)	0.06 (-0.10, 0.23)	0.04 (-0.13, 0.20)	0.17 (0.06, 0.29)**	0.22 (0.06, 0.38)**	0.15 (-0.02, 0.32)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.09 (-0.02, 0.19)	0.14 (-0.01, 0.28)	0.05 (-0.11, 0.20)	0.09 (-0.01, 0.20)	0.07 (-0.08, 0.22)	0.12 (-0.03, 0.27)	0.21 (0.10, 0.31)**	0.33 (0.19, 0.48)**	0.09 (-0.06, 0.25)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood blood pressure and heart rate in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use. *$p < 0.05$. ** $p < 0.01$.

Table 3. Associations of maternal psychological distress scales with childhood lipids profile at 10 years, total group and stratified for boys and girls[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Total Cholesterol			HDL Cholesterol			Triglycerides		
	Total group (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,873)	**Boys** (n=1,398)	**Girls** (n=1,475)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	-0.06 (-0.20, 0.08)	-0.05 (-0.24, 0.14)	-0.01 (-0.22, 0.20)	-0.09 (-0.23, 0.05)	-0.19 (-0.39, 0.00)	0.03 (-0.17, 0.24)	0.02 (-0.13, 0.16)	0.01 (-0.19, 0.21)	0.02 (-0.18, 0.22)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	-0.04 (-0.18, 0.10)	-0.14 (-0.34, 0.06)	0.12 (-0.09, 0.33)	-0.06 (-0.20, 0.09)	-0.17 (-0.38, 0.03)	0.08 (-0.13, 0.28)	0.04 (-0.11, 0.18)	0.02 (-0.19, 0.23)	0.06 (-0.14, 0.26)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	-0.01 (-0.14, 0.12)	0.03 (-0.15, 0.21)	-0.02 (-0.21, 0.18)	-0.09 (-0.22, 0.05)	-0.02 (-0.21, 0.17)	-0.15 (-0.33, 0.04)	0.17 (0.04, 0.30)*	0.01 (-0.18, 0.20)	0.35 (0.17, 0.53)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood lipids profile in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use. *$p < 0.05$. ** $p < 0.01$.

Table 4. Associations of maternal psychological distress scales with childhood glucose metabolism and inflammatory factors at 10 years, total group and stratified for boys and girls

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores[1]						Odds Ratio (95% CI)[2]		
	Insulin			Glucose			C-reactive protein (≥ 3mg/l)		
	Total group (n=2,878)	Boys (n=1,395)	Girls (n=1,483)	Total group (n=2,878)	Boys (n=1,397)	Girls (n=1,481)	Total group (n=2,882)	Boys (n=1,399)	Girls (n=1,483)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.03 (-0.11, 0.17)	0.06 (-0.13, 0.26)	0.02 (-0.19, 0.23)	-0.00 (-0.14, 0.14)	0.05 (-0.15, 0.24)	-0.08 (-0.29, 0.14)	1.25 (0.76, 2.07)	1.26 (0.57, 2.79)	1.33 (0.68, 2.58)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.08 (-0.07, 0.22)	0.11 (-0.09, 0.31)	0.05 (-0.15, 0.26)	-0.02 (-0.17, 0.12)	0.06 (-0.14, 0.26)	-0.13 (-0.34, 0.08)	1.09 (0.64, 1.85)	0.80 (0.32, 2.01)	1.38 (0.71, 2.69)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.06 (-0.08, 0.19)	0.05 (-0.14, 0.23)	0.09 (-0.10, 0.28)	0.04 (-0.09, 0.17)	0.13 (-0.05, 0.32)	-0.06 (-0.25, 0.13)	1.15 (0.69, 1.90)	0.77 (0.32, 1.89)	1.54 (0.83, 2.87)

[1] Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood glucose metabolism in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group.

[2] Values are odds ratios (95% confidence intervals) and represent the risk of childhood high C-reactive protein at 10 years for maternal overall distress, depression and anxiety compared to the reference group.

Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use. *$p < 0.05$. ** $p < 0.01$.

Maternal psychological distress and childhood lipids profile

In the unadjusted models, no associations were observed of any maternal psychological distress scales with total cholesterol concentrations. Overall psychological distress and depression were associated with lower HDL-cholesterol concentrations among boys, whereas anxiety was associated with lower HDL-cholesterol and higher triglycerides concentrations among girls (p-values<0.05) (**Table S4** in Supplementary Materials). After adjustment for potential confounders, only maternal anxiety remained associated with higher childhood triglycerides among girls (difference 0.35 (95% CI 0.17, 0.53) SDS) (**Table 3**). Similar associations were observed after further adjustment for body mass index at 10 years (**Table S5** in Supplementary Materials). No associations were observed of any maternal psychological distress scale with childhood LDL-cholesterol (**Table S6** in Supplementary Materials).

Maternal psychological distress and childhood glucose metabolism and inflammatory factors

Maternal overall psychological distress, depression and anxiety during pregnancy were associated with higher childhood insulin concentrations in the total group (p-values<0.05). Maternal depression was associated with higher childhood insulin concentrations among boys and girls, whereas anxiety was associated with higher childhood insulin concentrations among girls only (p-values<0.05). No associations were observed for childhood glucose concentrations. All maternal psychological distress scales were associated with an increased risk of high C-reactive protein concentrations among girls only (p-values<0.05). (**Table S7** in Supplementary Materials). The associations were no longer significant after adjustment for potential confounders (**Table 4**) and further adjustment for body mass index at 10 years (**Table S8** in Supplementary Materials).

DISCUSSION

In this population-based prospective cohort study, the associations of maternal psychological distress with childhood cardio-metabolic outcomes are largely explained by socio-economic and family-based factors. Maternal psychological distress, depression and anxiety during pregnancy were, independent of potential confounders, associated with higher childhood heart rate among boys. Maternal anxiety was also associated with higher triglycerides among girls. Maternal psychological distress was not associated with childhood blood pressure, cholesterol, insulin, glucose and C-reactive protein concentrations.

Interpretation of main findings

Maternal psychological distress during pregnancy may lead to fetal developmental adaptations, which programme cardio-metabolic disease of the offspring. [2] Previous studies

suggested an association between maternal distress during pregnancy and a higher risk of hypertension, insulin resistance, and type 2 diabetes in adolescence and adulthood, but not in childhood.[10-14, 25] Next to blood pressure, increased heart rate has been recognized as a risk factor for cardiovascular morbidity and mortality.[26] Previous studies reported that maternal stress during pregnancy is associated with higher fetal heart rate.[27, 28] We have previously described a positive association of maternal distress after pregnancy with infant heart rate, but no association was present for distress during pregnancy.[7] This latter study was performed in a subgroup of the current cohort. To our knowledge, no studies on the association between maternal psychological distress during pregnancy and lipids profile or inflammatory markers in childhood have been performed.

In the current study, the associations of maternal psychological distress, depression and anxiety with offspring blood pressure, cholesterol, insulin, glucose, or C-reactive protein concentrations seem to be explained by family based socio-demographic factors. However, independent of these factors, maternal overall psychological distress, depression and anxiety during pregnancy were associated with higher childhood heart rate at 10 years in boys, but not in girls. It has been proposed that fetal sex-specific placental responsiveness to maternal stress may result in increased risk for later diseases in boys. The higher growth rates of male fetuses may increase their vulnerability and subsequently place them at increased risk of adverse outcomes throughout the life course.[8] In the current study, we also observed that maternal anxiety, but not overall psychological distress and depression during pregnancy, was associated with higher triglycerides among girls. This suggests that the mechanisms relating maternal stress during pregnancy with childhood triglycerides may relate to specific psychological symptoms and be sex-specific. We cannot exclude the possibility of these results being a chance finding. We considered Bonferroni correction for multiple testing too strict since our outcomes are correlated.[29] However, the observed associations remained significant when considering a p-value of 0.017 (0.05/3 groups of outcomes). Altogether, our findings suggest that maternal psychological distress during pregnancy seems to have a small but persistent influence on cardio-metabolic profile during childhood.

We performed a model additionally adjusted for child body mass index, which might be in the causal pathway of the associations. Since the main results were similar with and without adjustment for child body mass index, the observed associations of maternal psychological distress with childhood heart rate and triglycerides concentrations seem to be independent of childhood adiposity. Fetal programming mechanisms might partly explain these associations. Fetal exposure to increased glucocorticoids levels due to adaptations of the maternal hypothalamic–pituitary–adrenal axis is the most well-known mechanism through which maternal psychological distress may influence the offspring cardio-metabolic outcomes.[4, 5] Another mechanism is the programming of the fetal autonomic nervous system, specifically changes in the balance of sympathetic and parasympathetic nervous system, by maternal psychological stress.[7] An elevated sympathetic nervous system activity established in

utero may affect fetal and childhood heart rate and subsequently may lead to cardiovascular diseases later in life. Further research is needed to identify the causality, the underlying mechanisms and to allow a better understanding of the sex-specific responses. Although the observed associations are small and without clinical relevance on individual level, the results may be important from a developmental perspective since cardio-metabolic risk factors tend to track into adulthood. Further studies are needed to replicate our findings and to assess the long-term cardio-metabolic consequences of maternal psychological distress.

Strengths and limitations

Strengths of this study were the prospective design, the large sample size and the detailed data available on childhood cardio-metabolic risk factors. This study also has limitations. We used all data available for each specific analysis in order to optimize statistical power. The analyses for childhood lipids profile, glucose metabolism and C-reactive protein may have lower statistical power due to lower sample sizes. Mothers of children with and without follow-up data were different regarding the socioeconomic background and prevalence of psychological distress. We cannot exclude the possibility of selection bias. We relied on a self-report questionnaire of maternal psychological distress, which might lead to misclassification bias, due to underreporting of psychological symptoms, and subsequently to underestimation of observed effects.[30] The use of non-fasting blood samples of childhood cardio-metabolic profile may have resulted in misclassification and thus may have led to underestimation of the observed associations. However, previous studies in adults have shown that non-fasting blood lipids levels can accurately predict increased risks of cardiovascular events later in life [31, 32] and that semi-fasted insulin resistance is moderately correlated with fasting values.[33] Finally, although we have adjusted for many sociodemographic and lifestyle variables known to influence the associations, residual confounding might still be an issue due to the observational design of the study.

CONCLUSIONS

The associations of maternal psychological distress with childhood cardio-metabolic outcomes are largely explained by socio-economic family factors. Maternal psychological distress may, independently of these factors, influence offspring heart rate and triglycerides concentrations. Promoting a healthy mental state during pregnancy may improve child cardio-metabolic health.

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SUPPLEMENTARY MATERIAL

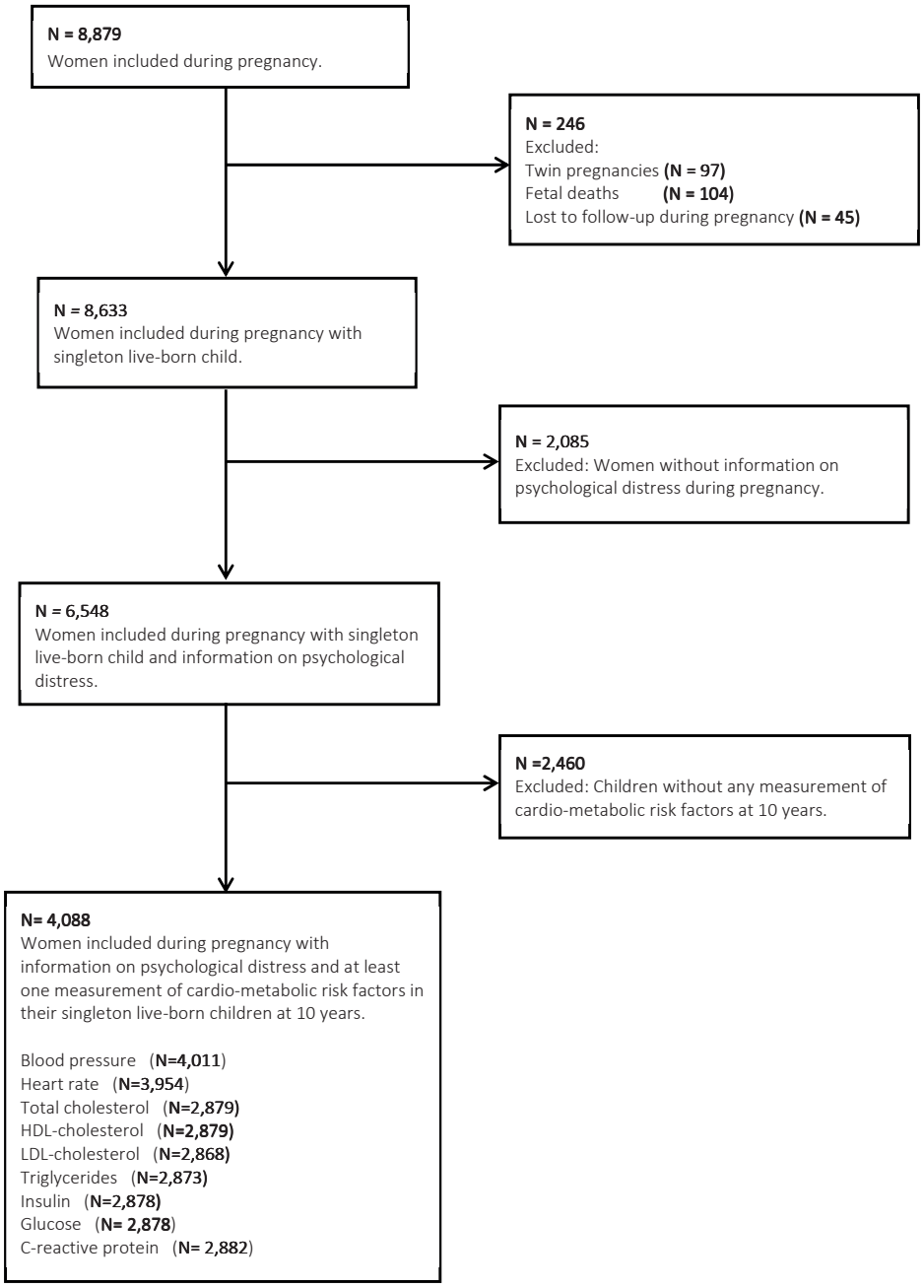

Figure S1. Flowchart of study population

Table S1. Comparison of maternal and child characteristics between mothers and children with and without follow-up data available[1]

	With follow-up (N= 4,088)	Without follow-up (N= 2,460)	P-value[2]
Maternal characteristics			
Age at intake, mean (SD), years	30.9 (4.8)	28.5 (5.4)	< 0.001
Ethnicity, N (%)			< 0.001
European	2,767 (68.2)	1,187 (50.5)	
Non-European	1,288 (31.8)	1,162 (49.5)	
Education, N (%)			< 0.001
Primary school	255 (6.4)	321 (14.1)	
Secondary school	1,628 (41.1)	1,196 (52.4)	
High education	2,076 (52.4)	764 (33.5)	
Marital status, N (%)			< 0.001
Married/living together	3,502 (89.2)	1,886 (82.4)	
No partner	425 (10.8)	404 (17.6)	
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.6 (18.1, 34.3)	22.6 (17.7, 35.1)	< 0.05
Alcohol consumption, N (%)			< 0.001
Yes	2,219 (59.9)	1,003 (45.5)	
No	1,486 (40.1)	1,200 (54.5)	
Smoking, N (%)			< 0.001
Yes	901 (24.0)	713 (31.8)	
No	2,847 (76.0)	1,531 (68.2)	
Folic acid supplement use, N (%)			< 0.001
No	650 (20.1)	648 (34.6)	
Start during first 10 weeks	1,030 (31.9)	598 (31.9)	
Preconceptional use	1,546 (47.9)	628 (33.5)	
Overall psychological distress, N (%)			< 0.001
Yes	352 (8.6)	358 (14.6)	
No	3,736 (91.4)	2,102 (85.4)	
Exposed to SSRIs, N (%)			< 0.001
Yes	43 (1.1)	37 (1.6)	
No	3,823 (98.9)	2,243 (98.4)	
Child characteristics			
Sex, N (%)			< 0.05
Boys	1,987 (48.6)	1,273 (51.7)	
Girls	2,101 (51.4)	1,187 (48.3)	
Gestational age at birth, N (%)			< 0.05
Preterm (< 37 weeks)	178 (4.4)	135 (5.5)	
Term (≥ 37 weeks)	3,910 (95.6)	2,325 (94.5)	
Birth weight[3], N (%)			< 0.05
Small for gestational age	405 (9.9)	263 (10.8)	

Table S1. Comparison of maternal and child characteristics between mothers and children with and without follow-up data available[1] (*continued*)

	With follow-up (N= 4,088)	Without follow-up (N= 2,460)	P-value[2]
Appropriate for gestational age	3,270 (80.1)	1,941 (79.8)	
Large for gestational age	409 (10.0)	227 (9.3)	
Age at visit, mean (SD), years	9.8 (0.3)	10.0 (0.8)	< 0.001
Body mass index, median (95% range), kg/m^2	16.9 (14.0, 24.5)	17.6 (13.4, 25.1)	< 0.05

[1] Values are means (standard deviation), medians (95% range) or numbers of subjects (valid %).

[2] P-values for differences in subject characteristics between groups were calculated performing independent sample t-tests for normally distributed continuous variables, Mann-Whitney tests for not normally distributed continuous variables and chi-square tests for categorical variables.

[3] Sex- and gestational age-adjusted birth weight SDS were created based on a North-European reference chart. Small and large size for gestational age at birth were defined as sex- and gestational age-adjusted birth weight below the 10th percentile and above the 90th percentile, respectively.

Table S2. Associations of maternal psychological distress scales with childhood blood pressure and heart rate at 10 years, total group and stratified for boys and girls (unadjusted models)[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Systolic blood pressure			Diastolic blood pressure			Heart rate		
	Total group (n=4,011)	**Boys** (n=1,945)	**Girls** (n=2,066)	**Total group** (n=4,011)	**Boys** (n=1,946)	**Girls** (n=2,065)	**Total group** (n=3,954)	**Boys** (n=1,918)	**Girls** (n=2,036)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.24 (0.13,0.35)**	0.31 (0.16, 0.46)**	0.18 (0.01, 0.34)*	0.20 (0.09,0.31)**	0.30 (0.15, 0.46)**	0.10 (-0.06, 0.26)	0.35 (0.24,0.46)**	0.47 (0.32, 0.62)**	0.25 (0.09, 0.41)**
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.16 (0.05,0.27)**	0.20 (0.05, 0.36)**	0.13 (-0.04, 0.29)	0.16 (0.05,0.27)**	0.24 (0.08, 0.39)**	0.09 (-0.07, 0.25)	0.28 (0.17,0.39)**	0.35 (0.20, 0.50)**	0.24 (0.08, 0.40)**
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.19 (0.08,0.29)**	0.26 (0.12, 0.41)**	0.12 (-0.03, 0.27)	0.18 (0.07,0.28)**	0.21 (0.06, 0.35)**	0.16 (0.01, 0.31)*	0.29 (0.18,0.40)**	0.43 (0.28, 0.57)**	0.17 (0.03, 0.32)*

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood blood pressure and heart rate in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Unadjusted models. *p < 0.05. ** p < 0.01.

Table S3. Associations of maternal psychological distress scales with childhood blood pressure and heart rate at 10 years, total group and stratified for boys and girls (body mass index adjusted models)[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Systolic blood pressure			Diastolic blood pressure			Heart rate		
	Total group (n=4,011)	**Boys** (n=1,945)	**Girls** (n=2,066)	**Total group** (n=4,011)	**Boys** (n=1,946)	**Girls** (n=2,065)	**Total group** (n=3,954)	**Boys** (n=1,918)	**Girls** (n=2,036)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.06 (-0.05, 0.17)	0.08 (-0.07, 0.23)	0.05 (-0.11, 0.21)	0.07 (-0.05, 0.18)	0.10 (-0.06, 0.26)	0.03 (-0.14, 0.20)	0.23 (0.11, 0.35)**	0.33 (0.17, 0.49)**	0.14 (-0.03, 0.31)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	-0.01 (-0.12, 0.10)	0.00 (-0.15, 0.15)	-0.01 (-0.17, 0.15)	0.04 (-0.07, 0.16)	0.06 (-0.11, 0.22)	0.03 (-0.14, 0.20)	0.17 (0.06, 0.29)**	0.22 (0.06, 0.37)**	0.15 (-0.02, 0.32)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.04 (-0.06, 0.14)	0.10 (-0.03, 0.24)	-0.01 (-0.16, 0.13)	0.08 (-0.03, 0.19)	0.06 (-0.09, 0.21)	0.11 (-0.05, 0.26)	0.20 (0.10, 0.31)**	0.33 (0.18, 0.47)**	0.10 (-0.06, 0.25)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood heart rate in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use and child body mass index. *$p < 0.05$. ** $p < 0.01$.

Table S4. Associations of maternal psychological distress scales with childhood lipids profile at 10 years, total group and stratified for boys and girls (unadjusted models)[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Total Chlesterol			HDL-Cholesterol			Triglycerides		
	Total group (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,873)	**Boys** (n=1,398)	**Girls** (n=1,475)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	-0.04 (-0.18, 0.09)	-0.00 (-0.19, 0.18)	-0.06 (-0.26, 0.14)	-0.17 (-0.30, -0.03)*	-0.28 (-0.47, -0.10)**	-0.07 (-0.26, 0.13)	0.09 (-0.04, 0.23)	0.13 (-0.06, 0.32)	0.07 (-0.12, 0.26)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	-0.03 (-0.16, 0.11)	-0.09 (-0.28, 0.09)	0.06 (-0.13, 0.26)	-0.12 (-0.26, 0.01)	-0.25 (-0.44, -0.06)*	-0.01 (-0.20, 0.18)	0.10 (-0.04, 0.23)	0.11 (-0.08, 0.31)	0.09 (-0.10, 0.28)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.00 (-0.13, 0.13)	0.05 (-0.13, 0.22)	-0.03 (-0.21, 0.16)	-0.14 (-0.27, -0.01)*	-0.11 (-0.29, 0.07)	-0.19 (-0.37,-0.01)*	0.22 (0.09, 0.35)**	0.09 (-0.09, 0.28)	0.37 (0.20, 0.55)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood lipids profile in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Unadjusted models.*p < 0.05. ** p < 0.01.

Table S5. Associations of maternal psychological distress scales with childhood lipids profile at 10 years, total group and stratified for boys and girls (body mass index adjusted models)[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Total Cholesterol			HDL-Cholesterol			Triglycerides		
	Total group (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,879)	**Boys** (n=1,397)	**Girls** (n=1,482)	**Total group** (n=2,873)	**Boys** (n=1,398)	**Girls** (n=1,475)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	-0.07 (-0.21, 0.08)	-0.06 (-0.25, 0.13)	-0.02 (-0.23, 0.20)	-0.09 (-0.23, 0.05)	-0.19 (-0.38, 0.00)	0.03 (-0.17, 0.23)	0.01 (-0.13, 0.15)	0.00 (-0.20, 0.20)	0.02 (-0.18, 0.22)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	-0.04 (-0.19, 0.10)	-0.15 (-0.34, 0.05)	0.12 (-0.09, 0.33)	-0.05 (-0.19, 0.09)	-0.17 (-0.37, 0.03)	0.09 (-0.11, 0.28)	0.03 (-0.11, 0.17)	0.01 (-0.19, 0.21)	0.06 (-0.14, 0.26)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	-0.02 (-0.15, 0.12)	0.03 (-0.15, 0.21)	-0.02 (-0.22, 0.17)	-0.07 (-0.20, 0.06)	-0.02 (-0.20, 0.16)	-0.11 (-0.29, 0.07)	0.16 (0.03, 0.29)*	0.00 (-0.18, 0.19)	0.32 (0.14, 0.50)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood lipids profile in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use and child body mass index. *$p < 0.05$. ** $p < 0.01$.

Table S6. Associations of maternal psychological distress scales with childhood LDL-cholesterol at 10 years, total group and stratified for boys and girls[1]

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores								
	Unadjusted models			Adjusted models			Body mass index adjusted models		
	Total group n=2,868)	**Boys** (n=1,394)	**Girls** (n=1,474)	**Total group** (n=2,868)	**Boys** (n=1,394)	**Girls** (n=1,474)	**Total group** (n=2,868)	**Boys** (n=1,394)	**Girls** (n=1,474)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	-0.00 (-0.14, 0.13)	0.10 (-0.08, 0.28)	-0.08 (-0.28, 0.11)	-0.02 (-0.17, 0.12)	0.06 (-0.13, 0.25)	-0.06 (-0.27, 0.15)	-0.03 (-0.17, 0.11)	0.05 (-0.14, 0.24)	-0.06 (-0.27, 0.15)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	-0.01 (-0.14, 0.13)	-0.00 (-0.19, 0.18)	0.02 (-0.18, 0.21)	-0.03 (-0.17, 0.12)	-0.04 (-0.24, 0.15)	0.05 (-0.16, 0.26)	-0.03 (-0.17, 0.12)	-0.05 (-0.24, 0.15)	0.05 (-0.16, 0.26)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	-0.02 (-0.14, 0.11)	0.08 (-0.09, 0.26)	-0.10 (-0.28, 0.09)	-0.03 (-0.17, 0.10)	0.06 (-0.12, 0.24)	-0.10 (-0.29, 0.10)	-0.04 (-0.18, 0.09)	0.06 (-0.12, 0.24)	-0.11 (-0.30, 0.08)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood LDL-cholesterol in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group.

Adjusted models include maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use.

Body mass index adjusted models additionally include child body mass index.

*p < 0.05. **p < 0.01.

Table S7. Associations of maternal psychological distress scales with childhood glucose metabolism and inflammatory factors at 10 years, total group and stratified for boys and girls (unadjusted models)

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores[1]						Odds Ratio (95% CI)[2]		
	Insulin			Glucose			C-reactive protein (≥ 3mg/l)		
	Total group (n=2,878)	Boys (n=1,395)	Girls (n=1,483)	Total group (n=2,878)	Boys (n=1,397)	Girls (n=1,481)	Total group (n=2,882)	Boys (n=1,399)	Girls (n=1,483)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.15 (0.02, 0.29)*	0.14 (-0.05, 0.32)	0.19 (-0.00, 0.39)	-0.02 (-0.15, 0.11)	0.03 (-0.15,0.22)	-0.09 (-0.28, 0.11)	2.04 (1.28, 3.24)**	1.79 (0.86, 3.72)	2.35 (1.28, 4.30)**
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.20 (0.06, 0.33)**	0.19 (0.01, 0.38)**	0.21 (0.02, 0.41)*	-0.04 (-0.17, 0.10)	0.05 (-0.14,0.23)	-0.13 (-0.33, 0.07)	1.77 (1.08, 2.89)*	1.17 (0.49, 2.78)	2.34 (1.28, 4.29)**
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.14 (0.01, 0.27)*	0.11 (-0.07, 0.29)	0.19 (0.01, 0.38)*	0.04 (-0.09, 0.16)	0.12 (-0.05, 0.30)	-0.06 (-0.25, 0.12)	1.62 (1.01, 2.62)*	1.02 (0.43, 2.41)	2.19 (1.22, 3.94)**

[1] Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood glucose metabolism in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group. Unadjusted models.

[2] Values are odds ratios (95% confidence intervals) and represent the risk of childhood high C-reactive protein at 10 years for maternal overall distress, depression and anxiety compared to the reference group. Unadjusted models.

*p < 0.05. ** p < 0.01.

Table S8. Associations of maternal psychological distress scales with childhood glucose metabolism and inflammatory factors at 10 years, total group and stratified for boys and girls (body mass index adjusted models)

Maternal psychological distress scales	Difference (95% CI) in standard deviation scores[1]						Odds Ratio (95% CI)[2]		
	Insulin			Glucose			C-reactive protein (≥ 3mg/l)		
	Total group (n=2,878)	Boys (n=1,395)	Girls (n=1,483)	Total group (n=2,878)	Boys (n=1,397)	Girls (n=1,481)	Total group (n=2,882)	Boys (n=1,399)	Girls (n=1,483)
Overall distress									
No stress	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Stress	0.03 (-0.11, 0.17)	0.06 (-0.13, 0.25)	0.03 (-0.17, 0.23)	0.00 (-0.14, 0.15)	0.05 (-0.15, 0.25)	-0.07 (-0.28, 0.14)	1.21 (0.72, 2.01)	1.22 (0.55, 2.72)	1.26 (0.63, 2.50)
Depression									
No depression	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Depression	0.07 (-0.07, 0.21)	0.11 (-0.09, 0.30)	0.05 (-0.15, 0.25)	-0.02 (-0.16, 0.13)	0.06 (-0.14, 0.26)	-0.11 (-0.32, 0.09)	1.07 (0.63, 1.83)	0.78 (0.31, 1.99)	1.34 (0.68, 2.65)
Anxiety									
No anxiety	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Anxiety	0.04 (-0.09, 0.17)	0.05 (-0.13, 0.23)	0.05 (-0.13, 0.24)	0.05 (-0.09, 0.18)	0.13 (-0.05, 0.32)	-0.04 (-0.23, 0.15)	1.07 (0.64, 1.78)	0.76 (0.31, 1.87)	1.33 (0.70, 2.54)

[1] Values are linear regression coefficients (95% confidence intervals) and reflect the change in childhood glucose metabolism in standard deviation scores for maternal overall distress, depression and anxiety, compared to the reference group.

[2] Values are odds ratios (95% confidence intervals) and represent the risk of childhood high C-reactive protein at 10 years for maternal overall distress, depression and anxiety compared to the reference group.

Models are adjusted for maternal age, ethnicity, educational level, marital status, body mass index before pregnancy, alcohol consumption, smoking during pregnancy, folic acid and selective serotonin reuptake inhibitors use and child body mass index. *$p < 0.05$. ** $p < 0.01$.

Chapter 2.3

Infant growth patterns, childhood body mass index and arterial health at age 10 years

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Adapted from: *Obesity (Silver Spring). 2022;30(3):770-8.*

ABSTRACT

Objective: Associations of obesity with cardiovascular disease may originate in childhood. This study examined critical periods for BMI in relation to arterial health at school age.

Methods: Among 4,731 children from a prospective cohort study, associations of infant peak weight velocity, both age and BMI at adiposity peak, and BMI trajectories with carotid artery intima-media thickness and carotid artery distensibility at 10 years were examined.

Results: A 1-standard deviation score (SDS) higher peak weight velocity and BMI at adiposity peak were associated with higher intima-media thickness (0.10 SDS; 95% CI: 0.06 to 0.13 and 0.08 SDS; 95% CI: 0.05 to 0.12) and lower distensibility (−0.07 SDS; 95% CI: −0.10 to −0.03 and −0.07 SDS; 95% CI: −0.11 to −0.03) at 10 years. For distensibility, current BMI explained these associations. Children within the highest BMI tertile at ages 2 and 10 years had the lowest distensibility ($p < 0.05$), but similar intima-media thickness, compared with children constantly within the middle tertile.

Conclusions: Infant weight growth patterns and childhood BMI are associated with subtle differences in carotid intima-media thickness and carotid distensibility at school age. For distensibility, current BMI seems critical. Follow-up is needed to determine whether these associations lead to adult cardiovascular disease.

INTRODUCTION

Obesity is a major risk factor for atherosclerotic cardiovascular disease in adults.[1, 2] Childhood adiposity, which tracks into adulthood, also is associated with cardiovascular risk factors and disease in adulthood.[3-7] Carotid intima-media thickness and distensibility are two measures of arterial structure and function, respectively. These markers for arterial health could be used to assess cardiovascular risk in children.[8, 9] Previous cross-sectional and prospective observational studies reported associations of higher body mass index (BMI) from school-age onwards with higher carotid intima-media thickness and lower carotid distensibility from school-age to adulthood.[5, 10-14] Furthermore, one study among 1,811 Australian adolescents reported that cumulative exposure to a high BMI from 2 years onwards was associated with a higher carotid intima-media thickness at age 12 years.[15] Another study among 500 Finnish adolescents reported that participants with relatively low brachial or aortic distensibility during adolescence had higher body mass indices from infancy onwards than those with high distensibility.[16] In the same cohort as the current study, we have previously shown that higher peak weight velocity and BMI at adiposity peak are strongly associated with an increased risk of childhood overweight and obesity, as well as with cardiovascular outcomes at school-age.[17-20] These studies strongly suggest that early-life weight growth patterns affect cardiovascular health in later life. Still, it is not yet known whether infant weight growth patterns or body mass trajectories across childhood are associated with higher carotid intima-media thickness and lower carotid distensibility at age 10 years. Identification of such associations is important from an etiologic perspective.

We hypothesized that a higher BMI from infancy across school-age is associated with carotid intima-media thickness and carotid distensibility. In a population-based cohort study among 4,731 children, we examined the associations of infant weight growth patterns and BMI trajectories from age 2 to 10 years with carotid intima-media thickness and carotid distensibility in children aged 10 years.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onward in Rotterdam, the Netherlands.[21] The Medical Ethical Committee of Erasmus Medical Center approved the study (MEC 198.782/2001/31). Pregnant women with an expected delivery date between April 2002 and January 2006 who were living in Rotterdam were eligible to participate. In this study, we included 4,731 singleton children with data on infant weight growth velocity patterns and/or BMI across childhood, and carotid intima-media thickness or carotid distensibility measured at the median age of 9.7 years (95%

CI: 9.4, 10.5). Written informed consent was provided by their parents. Supplementary Material **Figure S1** shows a flowchart of participants.

Growth measurements

We obtained information on repeated infant and pre-school (0 to 4 years) length and weight measurements from community health centers.[21] At ages 6 and 10 years, we invited children to our research facility for detailed measurements. We measured height and weight without shoes or heavy clothing, from which we calculated BMI (weight in kilograms divided by height in meters

squared) and, subsequently, sex- and age-adjusted standard-deviation-scores (SDS), based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[22] From infant growth measures, we derived peak weight velocity, reflecting the greatest weight change in infancy, and both age and BMI reached at adiposity peak, as described previously.[20, 23] Peak weight velocity was derived by fitting the Reed1 model by sex on all weight measurements taken between birth and age 3 years.[24] The first derivative of the fitted distance curve was taken to obtain the weight velocity curve. Peak weight velocity, reflecting the maximum rate of growth in infancy, was defined as the maximum of this curve. We obtained both age and BMI at adiposity peak by fitting a cubic mixed-effects model on log(BMI) from age 14 days to 1.5 years, adjusted for sex.[20] These measures refer to the age and BMI level, respectively, at which the infant reaches its maximum BMI change. We categorized children at median ages 2.1 (95% CI: 1.2-3.0), 6.0 (95% CI: 5.6, 7.3) and 9.7 (95% CI: 9.3, 10.5) years into BMI tertiles. Subsequently, to examine body mass growth pattern, we created three variables combining BMI index at ages 2 and 6 years; ages 6 and 10 years; and ages 2 and 10 years. Thus, these three variables reflected each nine different BMI combinations, across a different age interval. At age 10 years, we also categorized BMI into underweight, normal weight, overweight, and obesity based on the International Obesity Task Force cutoffs.[25]

Carotid intima-media thickness and carotid distensibility

When children visited the research facility at age 10 years, we measured intima-media thickness and distensibility three times at both common carotid arteries (n=5,746) using the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA) device. Children were in the supine position, with the head tilted slightly away from the transducer. The common carotid artery was identified in a longitudinal plane, ~10 mm proximal from the carotid bifurcation. We obtained six recordings that ideally included multiple heart cycles. The analyses were performed offline and semiautomatically, using the application Carotid Studio (Cardiovascular Suite; Quipu srl; Pisa; Italy). For each recording, at all R-waves of the simultaneous electrocardiogram (ECG), carotid intima-media thickness was computed at the far wall as the average distance between lumen-intima and media-adventitia borders. The average carotid intima-media thickness of all frames of the acquired image sequence was computed. The distensibility coefficient, or

distensibility, was defined as the relative change in lumen area during systole for a given peripheral pressure change. We assessed blood pressure at the right brachial artery four times with the validated automatic sphygmomanometer Datascope Accutorr Plus (Paramus, New Jersey).[26] The lumen diameter of the carotid artery was computed as the average distance between the far and near media-adventitia interfaces, for each frame of the acquired image sequence. Distension was calculated as the difference between the maximal (diastolic) and minimal (systolic) lumen diameter of the carotid artery. Per recording, the average distension and diameter values were used to compute the average carotid distensibility. During these offline analyses, we excluded 516 and 704 children, without any valid carotid intima-media thickness or carotid distensibility measurement, respectively; reasons included lack of appropriate recording, insufficient quality of the recording, recording of the heart only, or no blood pressure measurement available to calculate carotid distensibility. Further data processing for the remaining 5,230 and 5,042 children with carotid intima-media thickness and carotid distensibility data, respectively, was performed using R (The R Foundation, Vienna, Austria). We excluded 9 children with unreliable low or high carotid distensibility values. We used the overall mean carotid intima-media thickness (millimeters) and carotid distensibility (kPa^{-1} x 10^{-3}) as main outcomes of interest. In a reproducibility study among 47 participants, the interobserver and intraobserver intraclass correlation coefficients were >0.85.

Covariates

We constructed a directed acyclic diagram (**Figure S2** in Supplementary Materials). Potential covariates were selected based on previous literature and by observing a >10% change in effect estimate. We obtained information on maternal age, educational level, pre-pregnancy BMI, parity, folic acid supplement use, smoking and alcohol consumption during pregnancy, child ethnicity and breastfeeding from questionnaires.[21] From midwife and hospital records we obtained information on child sex and birth weight, for which we created sex- and gestational age-adjusted SDS.[27] At ages 6 and 10 years, blood pressure was assessed at the right brachial artery four times with the validated automatic sphygmomanometer Datascope Accutorr Plus. Mean systolic and diastolic blood pressure were calculated from the last three measurements.[26] Subsequently, mean arterial pressure was calculated using the following formula:

$$\text{Diastolic Blood Pressure} + \tfrac{1}{3} \times (\text{Systolic Blood Pressure} - \text{Diastolic Blood Pressure}).$$

Gestational diabetes did not change the results; therefore, it was not included in the model.

Statistical analysis

First, we performed a non-response analysis by comparing characteristics of children with and without carotid artery ultrasound data, using Student's t-tests, Mann-Whitney tests and Chi-square tests. Second, we examined the associations of infant peak weight velocity,

age at adiposity peak, and BMI at adiposity peak with carotid intima-media thickness and carotid distensibility using linear multivariable regression models. Third, to examine the associations of three BMI combinations across childhood (ages 2-6 years, ages 6-10 years, and ages 2-10 years) with carotid intima-media thickness and carotid distensibility, we used linear multivariable regression after adjustment for the age interval between exposure measurements. Fourth, we examined cross-sectional associations of BMI in categories with both outcomes. We examined trends using BMI continuously. Basic models were adjusted for sex and age at outcome measurement. Confounder models were considered as main models and additionally adjusted for ethnicity and birth weight SDS, maternal age, education, parity, BMI, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. For analyses of infant growth, we further explored significant associations ($p < 0.05$) in the confounder model by examining whether they were independent of BMI at age 10 years after excluding multicollinearity as a threat to the validity of these models (variance inflation factors ≤2.5). As sensitivity analyses, we additionally adjusted confounder models for mean arterial pressure, which we considered to be a potential mediator. To compare effect estimates, we analyzed exposures and outcomes in SDS, after natural log transformation of carotid distensibility, which had a skewed distribution. Interaction terms between exposures and birth weight SDS or sex in relation to both outcomes were not significant in the basic models ($p_{interaction} > 0.05$). The interaction term between BMI at age 10 years and ethnicity was significant for carotid intima-media thickness. Therefore, exploratory analyses of BMI with this outcome were performed among children from Dutch ethnic background, our largest subgroup (n=2,275). We used multiple imputations for covariates with missing values using the Markov Chain Monte Carlo method. We created five datasets and report pooled regression coefficients.[28] We performed statistical analyses using Statistical Package of Social Sciences version 25.0 (SPSS IBM, Chicago, Illinois, United States). As exposures were correlated, we did not correct for multiple testing and specify two-sided $p< 0.05$ and $p< 0.01$.

RESULTS

Subject characteristics

Table 1 and Supplementary **Table S1** show participant characteristics. At age 10 years, 74.9% of children had a normal BMI. The mean carotid intima-media thickness at this age was 0.46 (SD 0.04) mm and the median carotid distensibility was 55.8 (95% CI: 37.3, 85.4) $kPa^{-1}x\ 10^{-3}$. Compared with mothers of children with carotid artery ultrasound data, the mothers of children without these data were younger, had lower levels of education, and were multiparous. They also smoked more often but consumed alcohol or used folic acid supplements less frequently (**Table S2** in Supplementary Materials). Supplementary **Table S3** shows correlations between exposures and outcomes.

Table 1. Subject characteristics after imputation of covariates (n=4,731)[1]

	Values
Maternal characteristics	
Age, y	30.9 (5.0)
Educational level	
No, primary, secondary	2,442 (50.6)
College or higher	2,289 (49.4)
Parity	
Nulliparous	2,752 (58.2)
Multiparous	1,979 (41.8)
Pre-pregnancy body mass index, kg/m^2	22.8 (17.7, 34.0)
Smoking	
Non-smoker or smoked until pregnancy was known	4,016 (84.9)
Smoked throughout pregnancy	715 (15.1)
Alcohol consumption	
No consumption or consumption until pregnancy was known	2,719 (57.5)
Sustained consumption	2,012 (42.5)
Folic acid supplement use	
No	1,084 (22.9)
From early pregnancy	1,502 (32.8)
Yes, from preconception	2,145 (45.3)
Birth and infant characteristics	
Gestational age, wk	40.1 (35.4, 42.3)
Birth weight, kg	3.42 (0.57)
Sex	
Boy	2,348 (49.6)
Girl	2,383 (50.4)
Ethnicity	
European[2]	3,191 (67.4)
Non-European	1,540 (32.6)
Breastfeeding	
No	357 (7.5)
Yes	4,375 (92.5)
Childhood growth	
Age at peak weight velocity, mo	0.79 (0.18)
Peak weight velocity, kg/y	12.0 (8.6, 16.8)
Age at adiposity peak, mo	8.4 (7.8, 9.6)
Body mass index at adiposity peak, kg/m^2	17.6 (0.80)
Childhood characteristics	
At 2 years	
Age at visit, mo	24.8 (23.4, 28.2)
Body mass index, kg/m^2	16.5 (14.1, 19.6)
At 6 years	

Table 1. Subject characteristics after imputation of covariates (n=4,731)[1] (*continued*)

	Values
Age at visit, y	6.0 (5.6, 7.6)
Body mass index, kg/m^2	15.8 (13.6, 20.9)
At 10 years	
Age at visit, y	9.7 (9.4, 10.5)
Body mass index[3], kg/m^2	17.0 (14.0, 24.8)
Underweight	327 (6.9)
Normal weight	3537 (74.9)
Overweight	678 (14.4)
Obese	179 (3.8)
Common carotid artery intima-media thickness, mm	0.46 (0.04)
Common carotid artery distensibility[4], kPa^{-1}*10^{-3}	55.8 (37.1, 85.4)
Blood pressure, mmHg	
Systolic	103 (8)
Diastolic	59 (6)
Mean arterial pressure	74 (6)

[1] Exposures and outcomes were not imputed. **Table S1** shows values based on observed, not imputed data. Values are median (95% range), mean ± SD, or n (%).
[2] A subgroup of 2,775 children was from Dutch ethnic background and used for exploratory sensitivity analyses.
[3] Categorized based on the International Obesity Task Force cutoffs.[19]
[4] Indicate values before natural-log transformation.

Infant growth and markers of arterial health

Higher peak weight velocity and body mass index at adiposity peak were both associated with higher carotid intima-media thickness (0.10 SDS; 95% CI: 0.06-0.13 and 0.08 SDS; 95% CI: 0.05-0.12), respectively, per SDS increase in growth measure) and lower carotid distensibility (-0.07 SDS; 95% CI: -0.10 to -0.03 and -0.07 SDS; 95% CI: -0.11 to -0.03, respectively, per SDS) at age 10 years. The associations for carotid distensibility were fully explained by body mass index at age 10 years (**Table 2**). Age at adiposity peak was not associated with both outcomes. Basic models showed similar results (**Table S4** in Supplementary Materials).

Childhood BMI and markers of arterial health

Stratified analyses showed that, compared with children in the middle BMI tertile at ages 2 and 10 years, children in the highest tertile at both ages had the lowest carotid distensibility (difference −0.26 SDS; 95% CI: −0.38 to −0.14; **Table 3**). No consistent associations were observed for carotid intima- media thickness. Compared with children within the middle BMI tertile at ages 2 and 10 years, those within the lowest tertile at both ages had the lowest carotid intima-media thickness and highest distensibility (differences −0.18 SDS; 95% CI: −0.30 to −0.06 and 0.17 SDS; 95% CI: 0.05 to 0.29, respectively; **Table 3**). Basic models showed similar results (Supporting Informaton Table S5). Patterns for BMI change between ages 2 and 6 years and ages 6 and 10 years were similar (**Tables S6** and **S7** in Supplementary Materials).

Table 2. Associations of infant growth measures with carotid intima-media thickness and carotid distensibility at age 10 years[1]

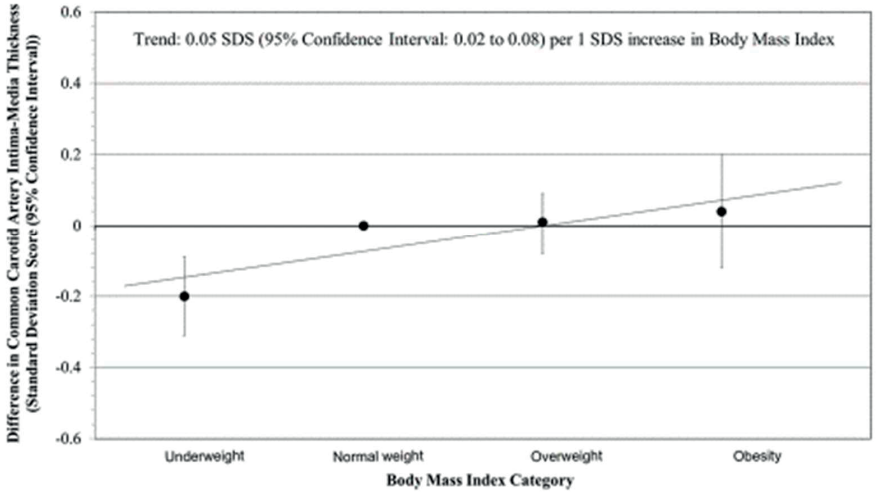
	Standard Deviation Scores, Regression Coefficients (95% CI)			
	Common carotid artery intima-media thickness n=3,779		Common carotid artery distensibility n=3,611	
	Confounder model	BMI model	Confounder model	BMI model
Peak weight velocity, SDS	0.10 (0.06, 0.13)**	0.09 (0.05, 0.13)**	-0.07 (-0.10, -0.03)**	-0.02 (-0.06, 0.02)
Age at adiposity peak, SDS	0.02 (-0.01, 0.05)	0.02 (-0.02, 0.05)	-0.02 (-0.05, 0.02)	-0.01 (-0.04, 0.03)
Body mass index at adiposity peak, SDS	0.08 (0.05, 0.12)**	0.07 (0.03, 0.11)**	-0.07 (-0.11, -0.03)**	-0.01 (-0.05, 0.03)

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Models were adjusted for child sex, age at outcome measurement, birth weight SDS, ethnicity, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. Body mass index models were additionally adjusted for child body mass index SDS at outcome measurement. ** $p < 0.01$.

Table 3. Associations of body mass index patterns across childhood with carotid intima-media thickness and carotid distensibility at age 10 years[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)			
	BMI at 10 years			
	First tertile	Second tertile	Third tertile	P_{trend}
Common carotid artery intima-media thickness (n=3,855)				
Body mass index 2 years				
First tertile	-0.18 (-0.30, -0.06)**	-0.06 (-0.20, 0.07)	-0.07 (-0.23, 0.09)	.16
	(n=694)	(n=363)	(n=228)	
Second tertile	-0.15 (-0.28, -0.02)*	*Reference*	-0.08 (-0.21, 0.06)	.34
	(n=421)	(n=479)	(n=385)	
Third tertile	-0.05 (-0.23, 0.12)	0.07 (-0.06, 0.20)	0.07 (-0.05, 0.19)	.94
	(n=170)	(n=443)	(n=672)	
P_{trend}	0.25	0.03	0.09	
Common carotid artery distensibility (n=3684)				
Body mass index 2 years				
First tertile	0.17 (0.05, 0.29)**	-0.02 (-0.16, 0.12)	-0.15 (-0.32, 0.01)	<.001
	(n=659)	(n=349)	(n=219)	
Second tertile	0.13 (-0.01, 0.26)	*Reference*	-0.22 (-0.36, -0.08)**	<.001
	(n=402)	(n=450)	(n=368)	
Third tertile	0.01 (-0.17, 0.19)	-0.15 (-0.28, -0.01)*	-0.26 (-0.38, -0.14)**	<.001
	(n=165)	(n=424)	(n=648)	
P_{trend}	0.11	0.09	0.15	

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Models were adjusted for child sex, age at outcome measurement, birth weight SDS, ethnicity, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. * $p < 0.05$. ** $p < 0.01$



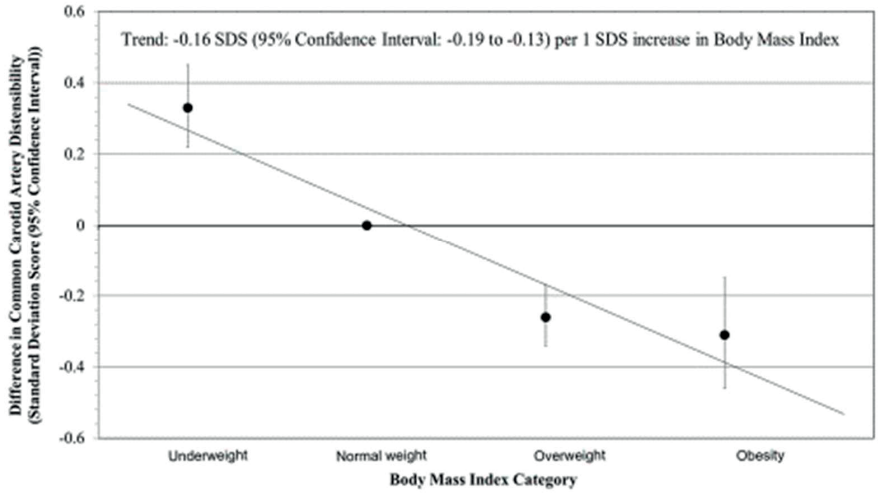


Figure 1. Associations of body mass index with carotid intima-media thickness and carotid distensibility at age 10 years[1]

[1]Values are regression coefficients (95% CI) from linear multivariable regression models that reflect differences in childhood carotid intima-media thickness (left panel, n=4,731) and log-transformed carotid distensibility (right panel, n=4,554), in standard deviation score, for each body mass index category as compared with the reference group (children with normal weight). Models were adjusted for child sex, age at outcome measurement, birth weight SDS, ethnicity, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. p for linear trend < 0.01.

At age 10 years, higher BMI was associated with higher carotid intima-media thickness (0.05 SDS = 95% CI: 0.02 to 0.08, per SDS) and lower carotid distensibility (−0.16 SDS; 95% CI: −0.19 to −0.13, per SDS; Figure 1). Compared with children with normal weight at age 10 years, those with underweight had lower carotid intima-media thickness (difference −0.23 SDS; 95% CI: −0.31 to −0.09) and higher carotid distensibility (difference 0.33 SDS; 95% CI: 0.22 to 0.45), whereas those with overweight and obesity had only lower carotid distensibility (differences −0.26 SDS; 95% CI: −0.34 to −0.17 and −0.31 SDS; 95% CI: −0.46 to −0.15, respectively). Basic models showed similar results (**Table S8** in Supplementary Materials).

Sensitivity analyses

The identified associations were largely similar after adjustment for mean arterial pressure (data not shown). Among Dutch children, we observed tendencies for similar associations with carotid intima-media thickness, although not significant, likely because of lower numbers (**Tables S9** and **S10** in Supplementary Materials).

DISCUSSION

In this large population-based cohort study of healthy children, we observed that both higher peak weight velocity and BMI at adiposity peak were associated with higher carotid intima-media thickness at age 10 years. Associations of these exposures with lower carotid distensibility were fully explained by BMI at outcome measurement. BMI across childhood was more consistently associated with carotid distensibility than with carotid intima-media thickness at age 10 years.

Interpretation of main findings

Early-life growth is associated with cardiovascular outcomes at school-age and cardiovascular disease in adulthood.[6, 17, 18, 29] Previous observational studies in children reported associations of higher body mass index with higher carotid intima-media thickness and lower carotid or brachial distensibility from school-age onwards.[5, 10-12, 14, 30, 31] Also, two previous studies have reported associations of repeated BMI measurements from infancy onwards with either carotid intima-media thickness or carotid distensibility in adolescents.[15, 16] We hypothesized that BMI from infancy onwards is associated with higher carotid intima-media thickness and lower carotid distensibility already at age 10 years. The identification of such associations is important from an etiological perspective.

Intima-media thickness may reflect early structural atherosclerotic changes within the intimal layer of arteries.[32, 33] Additionally, it may reflect physiological remodeling of the medial layer in response to growth.[14, 32] Higher intima-media thickness has been associated with cardiovascular disease in adults.[34] We were the first study, to our knowledge, that reported

on infant weight growth velocity patterns in relation to carotid intima-media thickness. We observed positive associations of infant peak weight velocity and BMI at adiposity peak with this measure at age 10 years. We also observed some evidence that BMI across childhood is positively associated with carotid intima-media thickness at age 10 years, although this may be restricted to lean children. Our findings seem in line with those from previous cross-sectional studies in adolescents and less often in children.[12-14, 35, 36] Of these, a large study among 3,497 children aged 6 to 17 years from five worldwide population-based studies, reported that children with overweight had higher carotid intima-media thickness than children with normal weight.[13] One prospective study from Australia among 1,811 healthy adolescents reported associations of obesity but not overweight from age 2 years onwards with a higher carotid intima-media thickness at age 12 years.[15] This smaller study among slightly older children, as compared to our population, also reported that cumulative exposure to a high BMI from age 2 years onwards was associated with a higher carotid intima-media thickness at age 12 years.[15] Therefore, our large prospective study adds to previous studies by reporting associations of detailed infant growth indices and of childhood BMI with higher carotid intima-media thickness in lean children aged 10 years.

The distensibility coefficient reflects the elastic properties of arteries as hollow structures.[37] It depends on the elastin-to-collagen-protein ratio in the extracellular matrix of the medial layer.[16, 38] Lower arterial distensibility has been associated with cardiovascular disease in adults.[39] We observed that infant peak weight velocity and BMI at adiposity peak were inversely associated with carotid distensibility at age 10 years. These associations were explained by BMI at age 10 years, underscoring the importance of weight management across childhood. We also observed that body mass growth across childhood was inversely associated with carotid distensibility at age 10 years. In contrast to carotid intima-media thickness, this finding was not restricted to lean children. Our results suggest that suboptimal BMI in children may be associated with early functional changes of the carotids.[33, 38] Previous cross-sectional European studies, among 65 up to 838 children or adolescents, reported either null findings or in line with ours, i.e., inverse associations of BMI with distensibility.[12, 14, 30, 36] One prospective study, among up to 500 Finnish healthy adolescents with data on carotid and aortic distensibility measurements between ages 11 and 19 years, reported that adolescents with a mean arterial distensibility below the 20th percentile of the study population had higher BMI from infancy onwards than adolescents with values above this cut-off.[16] Contrary to this smaller study, we assessed school-aged children with data on infant weight growth velocity patterns and analyzed distensibility continuously after detailed adjustment for covariates. The Finnish study further reported an inverse association of repeated BMI measurements between ages 11 and 15 years with aortic, but not carotid distensibility at these ages, whereas similar analyses between ages 15 and 19 years showed the converse.[16] Thus, our findings in a larger sample suggest that associations of BMI with carotid distensibility are already pres-

ent at age 10 years. Overall, we showed for the first time that BMI from infancy onwards is negatively associated with carotid distensibility in healthy children aged 10 years.

The mechanisms underlying the observed associations are not known; therefore, we can only speculate about their interpretation.[40] Moreover, we cannot distinguish whether subtle differences in carotid intima-media thickness and carotid distensibility in relation to BMI represent preclinical pathological changes or physiological adaptations in response to normal growth. Assuming pathological changes, it may be that metabolic complications associated with obesity, such as insulin resistance, inflammation and higher blood pressure, mediate the identified associations. Also, adipose cells are metabolically active and produce leptin. This hormone regulates appetite and body weight and is involved in vascular physiology. It has angiogenetic activity, increases oxidative stress in endothelial cells and promotes vascular cell calcification and smooth muscle cell proliferation and migration.[41] Atherosclerosis and arterial stiffening are distinct but synergistic processes that often coexist and share risk factors.[38] Arterial stiffening seems to activate pathophysiologic mechanisms involved in atherogenesis.[16, 38] Our findings were more consistent for carotid distensibility than for carotid intima-media thickness, suggesting that functional changes proceed structural changes.[33] The observed associations may also be explained by normal growth. BMI is the sum of lean and fat mass index but cannot distinguish between these components. Lean mass is metabolically more active than fat mass and the main determinant of resting energy expenditure.[9] Thus, lean mass increases oxygen demand, which requires a higher cardiac output and thereby increases blood pressure.[32] Blood pressure has been linked to higher intima-media thickness and lower distensibility.[11, 16, 30] Lean mass might be a stronger determinant of cardiovascular structure and function than BMI in children.[14, 32, 42] Although more extreme values may be pathological, in healthy school-aged children, subtle differences in intima-media thickness an carotid distensibility may reflect adaptation to lean mass and blood pressure.[14, 32] This needs further study.

The effect estimates of the observed associations were small and may not be relevant at an individual level. Also, we observed that, compared with children with normal weight, those with overweight or obesity had lower carotid distensibility but similar carotid intima-media thickness. We did expect that overweight was associated with higher carotid intima-media thickness. Our findings suggest that the associations of BMI with measures of arterial health are complex and might be different for distensibility and intima-media thickness. The specific age at which BMI becomes associated with higher intima-media thickness should be further studied. Previously, childhood overweight that normalizes in adulthood has been associated with the same risk of cardiovascular risk factors in adulthood, compared with having a healthy BMI across life. [5] Obesity also tracks from childhood to adulthood.[3, 4] Therefore, on a population-based level, our findings underline the importance of a healthy BMI from infancy onwards.

Strengths and limitations

Major strengths of this study are its population-based prospective design, repeated BMI measurements and detailed outcome measurements. This study also had limitations. As included children were from an affluent background and predominantly lean, our findings may not be generalizable to the general population with a higher prevalence of obesity. Also, we had no data on infant BMI rebound available because of infrequent measurements. This measure may also be associated with arterial health. Furthermore, although BMI is a common screening tool that has a high sensitivity to identify childhood adiposity, it has a moderate specificity.[43] Therefore, some children in our population with normal BMI may, in fact, have excess adiposity. We calibrated carotid distensibility to brachial mean arterial pressure, which will have shifted the calculated distensibility to higher values.[44] Although we demonstrated high reproducibility, we cannot exclude observer bias in the carotid measurements. Last, we had data on many covariates, but information on diet and physical activity was not available at all ages. Therefore, the observed associations were not adjusted for these potential time-varying confounders and residual confounding might be an issue, as in any observational study.

CONCLUSION

Our findings suggest that in predominantly lean children, infant weight growth patterns and BMI across childhood are associated with subtle differences in carotid intima-media thickness and carotid distensibility at age 10 years. Our findings also underscore the importance of weight management across childhood, as, for carotid distensibility, the associations were dependent on BMI at outcome measurement. Whether the observed associations predispose children to increased risk of cardiovascular disease in later life needs further study.

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SUPPLEMENTARY MATERIAL

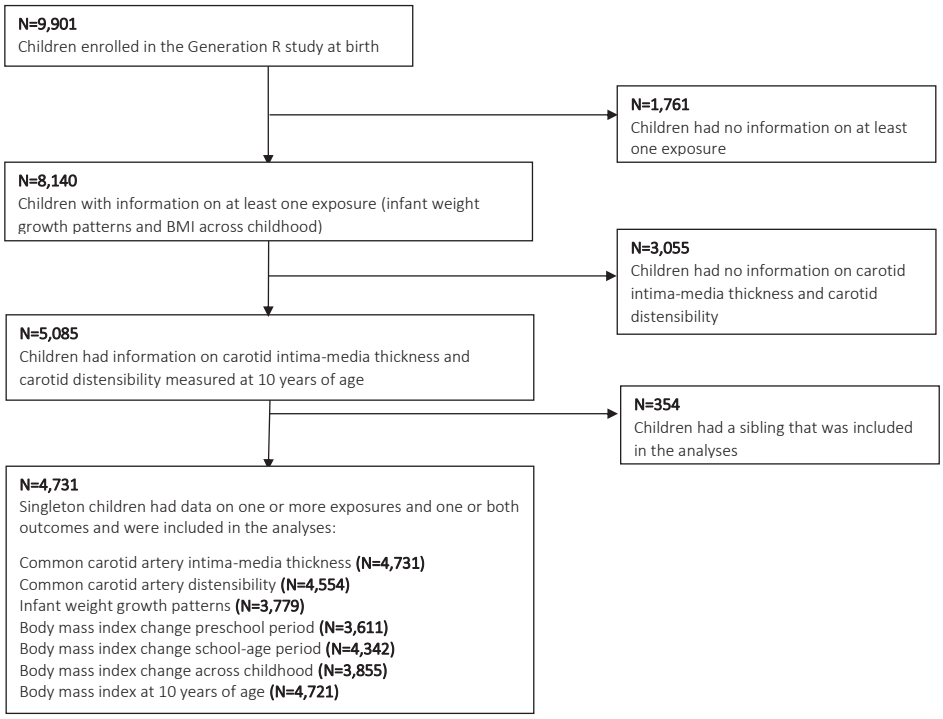

Figure S1. Flowchart of study population

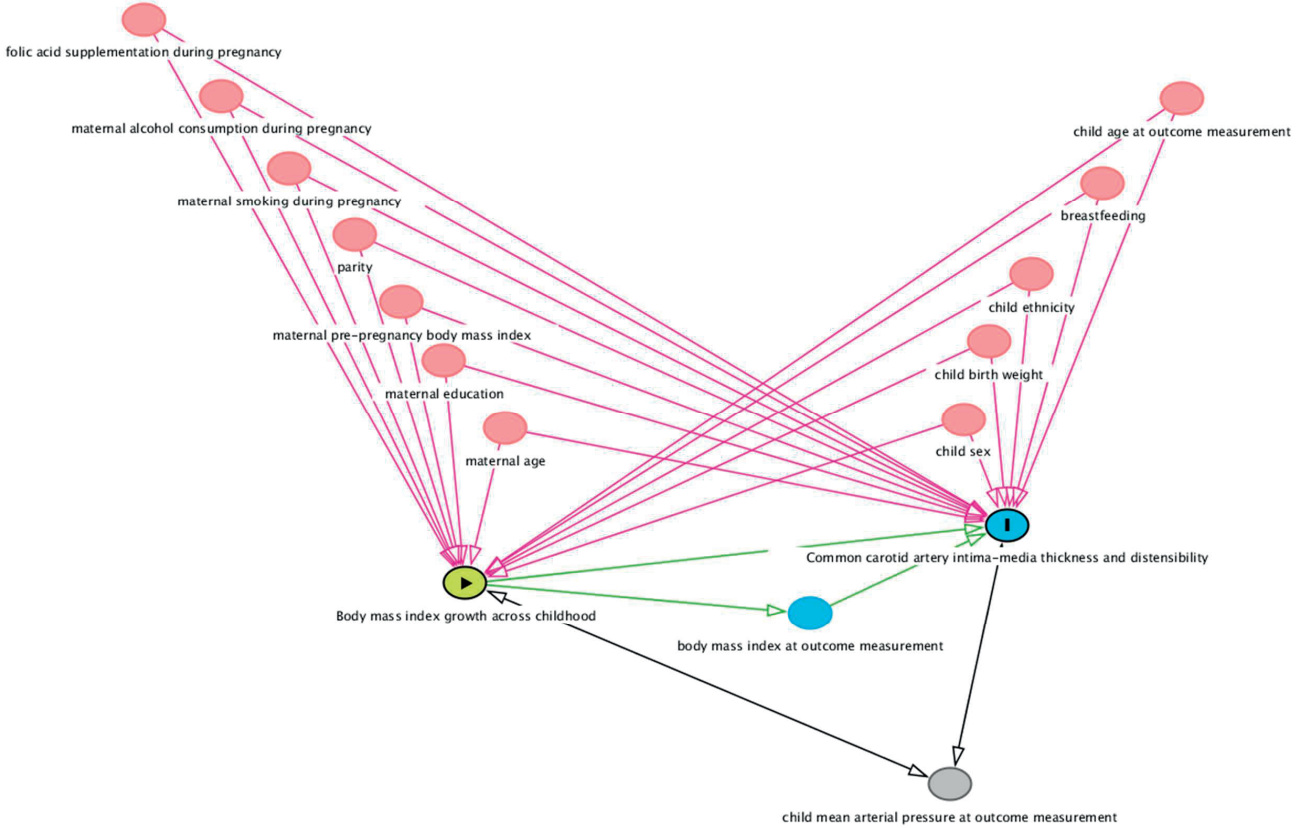

Figure S2. Directed acyclic graph

Directed acyclic graph represents assumptions of a causal relationship between body mass index across childhood and common carotid artery intima-media thickness and common carotid artery distensibility in late childhood. The other variables are considered confounders, a potential mediator, or, in case of blood pressure, a variable that is part of the same pathway.

Table S1. Participants characteristics based on observed, not imputed data (n=4731)[1]

	Values
Maternal characteristics	
Age, y	30.9 (5.0)
Educational level	
No, primary, secondary	2,200 (50.7)
College or higher	2,142 (49.3)
Parity	
Nulliparous	2,672 (58.3)
Multiparous	1,909 (41.7)
Pre-pregnancy body mass index, kg/m^2	22.6 (18.1, 34.7)
Smoking	
Non-smoker or smoked until pregnancy was known	3,512 (84.9)
Smoked throughout pregnancy	623 (15.1)
Alcohol consumption	
No consumption or consumption until pregnancy was known	2,195 (57.6)
Sustained consumption	1,615 (42.4)
Folic acid supplement use	
No	726 (22.0)
From early pregnancy	1,061 (32.2)
Yes, from preconception	1,509 (45.8)
Birth and infant characteristics	
Gestational age, wk	40.1 (35.4, 42.3)
Birth weight, kg	3.42 (0.57)
Sex	
Boy	2,348 (49.6)
Girl	2,383 (50.4)
Ethnicity	
European[2]	3,128 (67.7)
Non-European	1,495 (32.3)
Breastfeeding	
No	278 (7.4)
Yes	3,465 (92.6)
Childhood growth	
Age at peak weight velocity, mo	0.79 (0.18)
Peak weight velocity, kg/y	12.0 (8.6, 16.8)
Age at adiposity peak, m	8.4 (7.8, 9.6)
Body mass index at adiposity peak, kg/m^2	17.6 (0.80)
Childhood characteristics	
At 2 years	
Age at visit, mo	24.8 (23.4, 28.2)
Body mass index, kg/m^2	16.5 (14.1, 19.6)

Table S1. Participants characteristics based on observed, not imputed data (n=4731)[1] (*continued*)

	Values
At 6 years	
Age at visit, y	6.0 (5.6, 7.6)
Body mass index, kg/m^2	15.8 (13.6, 20.9)
At 10 years	
Age at visit, y	9.7 (9.4, 10.5)
Body mass index[3], kg/m^2	17.0 (14.0, 24.8)
Underweight	327 (6.9)
Normal weight	3,537 (74.9)
Overweight	678 (14.4)
Obese	179 (3.8)
Common carotid artery intima-media thickness, mm	0.46 (0.04)
Common carotid artery distensibility[4], kPa^{-1}*$^{10-3}$	55.8 (37.1, 85.4)
Blood pressure, mmHg	
Systolic	103 (8)
Diastolic	59 (6)
Mean arterial pressure	74 (6)

[1]Values after imputation of covariates are shown in **Table 1** of the manuscript. Values are median (95% range), mean ± SD, or n (%).

[2] A subgroup of 2775 children was from Dutch ethnic background and used for exploratory sensitivity analyses.

[3] Categorized based on the International Obesity Task Force cutoffs.[1]

[4] Indicate values before natural-log transformation.

Table S2. Non-response analysis (n=7,786)[1]

	Outcome (n=4,731)	No outcome (n=3,055)	P-value[2]
Maternal characteristics			
Age, y	30.9 (5.0)	29.4 (5.4)	<0.001
Educational level			
No, primary, secondary	2200 (50.7)	1606 (60.5)	<.0001
College or higher	2142 (49.3)	1047 (39.5)	
Parity			
Nulliparous	2672 (58.3)	1530 (52.1)	<0.001
Multiparous	1909 (41.7)	1407 (47.9)	
Pre-pregnancy body mass index, kg/m^2	22.6 (18.1, 34.7)	22.6 (17.9, 35.0)	0.44
Smoking			
Non-smoker or smoked until pregnancy was known	3512 (84.9)	2061 (80.0)	<0.001
Smoked throughout pregnancy	623 (15.1)	516 (20.0)	
Alcohol consumption			
No consumption or consumption until pregnancy was known	2195 (57.6)	1589 (67.0)	<0.001
Sustained consumption	1615 (42.4)	783 (33.0)	
Folic acid supplement use			
No	726 (22.0)	650 (32.0)	<0.001
From early pregnancy	1061 (32.2)	615 (33.0)	
Yes, from preconception	1509 (45.8)	766 (37.7)	
Birth and infant characteristics			
Gestational age, wk	40.1 (35.4, 42.3)	40.0 (35.6, 42.3)	0.22
Birth weight, kg	3.42 (0.57)	3.4 (0.56)	0.20
Sex			
Boy	2348 (49.6)	1574 (51.5)	0.10
Girl	2383 (50.4)	1481 (48.5)	
Ethnicity			
European	3128 (67.7)	1612 (57.2)	<0.001
Non-European	1495 (32.3)	1207 (42.8)	
Breastfeeding			
No	278 (7.4)	239 (9.9)	<0.05
Yes	3465 (92.6)	2179 (90.1)	
Peak weight velocity, kg/y	12.0 (8.6, 16.8)	12.3 (8.7, 17.4)	<0.001
Age at adiposity peak, m	8.4 (7.8, 9.6)	8.4 (7.8, 9.6)	0.07
Body mass index at adiposity peak, kg/m^2	17.6 (0.80)	17.6 (0.80)	0.01
Childhood characteristics			
At 2 years			
Age at visit, m	24.8 (23.4, 28.2)	24.7 (23.4, 28.1)	<0.05
Body mass index, kg/m^2	16.5 (14.1, 19.6)	16.5 (14.1, 19.6)	0.31
At 6 years			

Table S2. Non-response analysis (n=7,786)[1] (*continued*)

	Outcome (n=4,731)	No outcome (n=3,055)	P-value[2]
Age at visit, y	6.0 (5.6, 7.6)	6.1 (5.6, 7.7)	0.93
Body mass index, kg/m^2	15.8 (13.6, 20.9)	15.9 (13.6, 21.9)	<0.05
At 10 years			
Age at visit, y	9.7 (9.4, 10.5)	9.8 (9.4, 11.8)	<0.001
Body mass index[3], kg/m^2	17.0 (14.0, 24.8)	16.9 (14.1, 25.1)	0.84
Underweight	327 (6.9)	41 (6.7)	
Normal weight	3537 (74.9)	446 (72.8)	
Overweight	678 (14.4)	106 (17.3)	
Obese	179 (3.8)	20 (3.3)	
Blood pressure, mmHg			
Systolic	103 (8)	101 (8)	<0.001
Diastolic	59 (6)	57 (7)	<0.001
Mean arterial pressure	74 (6)	72 (7)	<0.001

[1]Values are based on observed, non-imputed data. Values are median (95% range), mean ± SD, or n (%).
[2]Differences in subject characteristics between children with and without outcome data were examined using Student's t-tests, Mann-Whitney tests and Chi-square tests.
[3]Categorized based on the International Obesity Task Force cutoffs.

Table S3. Correlation matrix of exposures and outcomes[1]

	Peak weight velocity	Age at adiposity peak	BMI at adiposity peak	BMI at 2 years	BMI at 6 years	BMI at 10 years	Mean arterial pressure at 10 years	Carotid intima-media thickness at 10 years	Carotid distensibility at 10 years
Peak weight velocity	1.000	-0.022	0.672**	0.406**	0.323**	0.228**	0.065**	0.126**	-0.109**
Age at adiposity peak		1.000	-0.226**	0.143**	0.027	-0.004	0.247	-0.001	-0.009
BMI at adiposity peak			1.000	0.627**	0.461**	0.306**	0.009	0.129**	-0.109**
BMI at 2 years				1.000	0.589**	0.420**	0.030	0.081**	-0.114**
BMI at 6 years					1.000	0.821**	0.193**	0.063**	-0.139**
BMI at 10 years						1.000	0.274**	0.041**	-0.163**
Mean arterial pressure at 10 years							1.000	0.050**	-0.123**
Carotid intima-media thickness at 10 years								1.000	-0.168**
Carotid distensibility 10 years									1.000

[1]Values reflect Pearson's correlation coefficients. *p < 0.05. ** p < 0.01.

Table S4. Associations of infant weight growth measures with carotid intima-media thickness and carotid distensibility at age 10 years (basic models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)	
	Common carotid artery intima-media thickness n=3,779	**Common carotid artery distensibility n=3,611**
Peak weight velocity, SDS	0.09 (0.05, 0.12)**	-0.07 (-0.11, -0.04)**
Age at adiposity peak, SDS	0.00 (-0.03, 0.03)	-0.01 (-0.04, 0.02)
BMI at adiposity peak, SDS	0.10 (0.07, 0.13)**	-0.08 (-0.12, -0.05)**

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Basic models were adjusted for child sex and age at outcome measurement. *p < 0.05. ** p < 0.01.

Table S5. Associations of body mass index patterns across childhood with carotid intima-media thickness and carotid distensibility at age 10 years (basic models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)					
	Common carotid artery intima-media thickness n=3,855			**Common carotid artery distensibility n=3,684**		
	BMI at 10 years					
	First tertile	Second tertile	Third tertile	First tertile	Second tertile	Third tertile
BMI at 2 years						
First tertile	(n=694)	(n=363)	(n=228)	(n=659)	(n=349)	(n=219)
	-0.19 (-0.31, -0.08)**	-0.08 (-0.21, 0.06)	-0.10 (-0.25, 0.06)	0.19 (0.07, 0.31)**	-0.02 (-0.16, 0.12)	-0.15 (-0.31, 0.01)
Second tertile	(n=421)	(n=479)	(n=385)	(n=402)	(n=450)	(n=368)
	-0.15 (-0.28, -0.02)*	*Reference*	-0.09 (-0.22, 0.04)	0.14 (0.01, 0.27)*	*Reference*	-0.22 (-0.36, -0.09)**
Third tertile	(n=170)	(n=443)	(n=672)	(n=165)	(n=424)	(n=648)
	-0.05 (-0.22, 0.13)	0.09 (-0.04, 0.21)	0.05 (-0.07, 0.16)	0.02 (-0.16, 0.20)	-0.14 (-0.27, -0.01)*	-0.28 (-0.40, -0.16)**

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Basic models were adjusted for child sex and age at outcome measurement. *p < 0.05. ** p < 0.01.

Table S6. Associations of body mass index patterns during the preschool period with carotid intima-media thickness and carotid distensibility at age 10 years (basic and confounder models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)					
	Common carotid artery intima-media thickness n=3,611			Common carotid artery distensibility n=3,447		
	BMI at 6 years					
	First tertile	Second tertile	Third tertile	First tertile	Second tertile	Third tertile
BMI at 2 years						
First tertile	(n=778)	(n=315)	(n=109)	(n=732)	(n=305)	(n=106)
Basic model	-0.14 (-0.25, -0.04)*	-0.05 (-0.19, 0.09)	0.05 (-0.15, 0.25)	0.16 (0.05, 0.27)**	0.03 (-0.12, 0.17)	-0.10 (-0.31, 0.11)
Confounder model	-0.13 (-0.24, -0.02)*	-0.03 (-0.17, 0.11)	0.08 (-0.13, 0.28)	0.15 (0.04, 0.26)*	0.03 (-0.11, 0.17)	-0.09 (-0.30, 0.12)
Second tertile	(n=342)	(n=532)	(n=331)	(n=321)	(n=511)	(n=312)
Basic model	-0.12 (-0.25, 0.02)	Reference	0.01 (-0.12, 0.15)	0.15 (0.01, 0.29)*	Reference	-0.17 (-0.31, -0.03)*
Confounder model	-0.11 (-0.24, 0.02)	Reference	0.02 (-0.11, 0.16)	0.14 (-0.00, 0.28)	Reference	-0.16 (-0.30, -0.02)*
Third tertile	(n=83)	(n=357)	(n=764)	(n=78)	(n=343)	(n=739)
Basic model	0.06 (-0.17, 0.29)	0.09 (-0.04. 0.23)	0.09 (-0.02, 0.20)	0.13 (-0.10. 0.37)	-0.13 (-0.26, 0.01)	-0.24 (-0.35, -0.12)**
Confounder model	0.05 (-0.18, 0.28)	0.09 (-0.05, 0.22)	0.09 (-0.02, 0.20)	0.13 (-0.11, 0.37)	-0.13 (-0.27, 0.01)	-0.22 (-0.33, -0.10)**

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Models were adjusted for child sex and age at outcome measurement. The confounder model was additionally adjusted for birth weight SDS, ethnicity, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. *$p < 0.05$. ** $p < 0.01$.

Table S7. Associations of body mass index patterns during the school-age period with carotid intima-media thickness and carotid distensibility at age 10 years (basic and confounder models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)					
	Common carotid artery intima-media thickness n=4,342			Common carotid artery distensibility n=4,175		
	BMI at 10 years					
	First tertile	Second tertile	Third tertile	First tertile	Second tertile	Third tertile
BMI at 6 years						
First tertile	(n=1,060)	(n=328)	(n=59)	(n=1,010)	(n=308)	(n=54)
Basic model	-0.23 (-0.32, -0.14)**	-0.14 (-0.27, -0.01)*	-0.19 (-0.46, 0.07)	0.23 (0.14, 0.33)**	0.02 (-0.11, 0.15)	0.05 (-0.22, 0.32)
Confounder model	-0.22 (-0.31, -0.12)**	-0.11 (-0.24, 0.01)*	-0.17 (-0.43, 0.09)	0.22 (0.13, 0.32)**	0.01 (-0.12, 0.14)	0.03 (-0.24, 0.30)
Second tertile	(n=355)	(n=776)	(n=317)	(n=349)	(n=743)	(n=308)
Basic model	-0.16 (-0.28, -0.03)*	*Reference*	-0.16 (-0.29, -0.03)*	0.16 (0.03. 0.28)*	*Reference*	-0.13 (-0.26, 0.00)*
Confounder model	-0.16 (-0.28, -0.03)*	*Reference*	-0.14 (-0.27, -0.01)*	0.15 (0.02, 0.27)*	*Reference*	-0.13 (-0.26, 0.00)*
Third tertile	(n=32)	(n=344)	(n=1,071)	(n=31)	(n=332)	(n=1,040)
Basic model	0.10 (-0.25, 0.44)	-0.00 (-0.13, 0.12)	-0.05 (-0.14, 0.04)	-0.12 (-0.47, 0.24)	-0.06 (-0.19, 0.07)	-0.23 (-0.32, -0.13)**
Confounder model	0.08 (-0.27, 0.43)	-0.02 (-0.14, 0.11)	-0.04 (-0.13, 0.06)	-0.11 (-0.46, 0.25)	-0.06 (-0.19, 0.07)	-0.22 (-0.31, -0.12)**

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. Models were adjusted for child sex and age at outcome measurement. The confounder model was additionally adjusted for birth weight SDS, ethnicity, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. *p < 0.05. ** p < 0.01.

Table S8. Cross-sectional associations body mass index with carotid intima-media thickness and carotid distensibility at age 10 years (basic models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)	
	Common carotid artery intima-media thickness n=4,731	Common carotid artery distensibility n=4,554
Body mass index		
Continuously, per 1 SDS	0.05 (0.03, 0.08)**	-0.16 (-0.19, -0.14)**
Categorized		
Underweight	-0.22 (-0.33, -0.11)**	0.36 (0.24, 0.47)**
Normal weight	*Reference*	*Reference*
Overweight	0.01 (-0.07, 0.10)	-0.27 (-0.36, -0.19)**
Obesity	0.04 (-0.11, 0.19)	-0.35 (-0.50, -0.20)**

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness and log-transformed carotid distensibility. The basic model was adjusted for child sex and age at outcome measurement. *p < 0.05. ** p < 0.01.

Table S9. Associations of body mass index patterns across childhood with carotid intima-media thickness at age 10 years in Dutch children (confounder models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)			
	BMI at 10 years			
	First tertile	Second tertile	Third tertile	P_{trend}
BMI at 2 years				
First tertile	-0.14 (-0.28, 0.00)	-0.13 (-0.30, 0.04)	-0.13 (-0.32, 0.07)	.17
	(n=431)	(n=221)	(n=150)	
Second tertile	-0.10 (-0.27, 0.06)	*Reference*	-0.08 (-0.25, 0.08)	.75
	(n=259)	(n=305)	(n=239)	
Third tertile	0.01 (-0.21, 022)	0.07 (-0.09, 0.22)	0.06 (-0.08, 0.21)	.68
	(n=112)	(n=227)	(n=413)	
P_{trend}	.41	**.004**	.12	

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness. The confounder model was adjusted for child sex, age at outcome measurement, birth weight SDS, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding.

Table S10. Cross-sectional associations of body mass index with carotid intima-media thickness at age 10 years in Dutch children (confounder models)[1]

	Standard Deviation Scores, Regression Coefficients (95% CI)
	Common carotid artery intima-media thickness n=2,775
Body mass index	
Continuously, per 1 SDS	0.04 (0.00, 0.08)*
Categorized	
Underweight	-0.23 (-0.37, -0.09)**
Normal weight	*Reference*
Overweight	-0.02 (-0.15, 0.10)
Obesity	-0.16 (-0.43, 0.11)

[1]Regression coefficients are linear multivariable regression coefficients based on standard deviation scores of carotid intima-media thickness. The confounder model was adjusted for child sex, age at outcome measurement, birth weight SDS, maternal age, education, parity, pre-pregnancy body mass index, folic acid supplementation, smoking and alcohol consumption during pregnancy and breastfeeding. *p < 0.05. ** p < 0.01.

Chapter 2.4

Endocrine disrupting chemicals exposures, body fat measures, and cardiovascular risk factors in school-age children

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Adapted from: *Obesity (Silver Spring). 2021;29(2):409-17.*

ABSTRACT

Objective: The purpose of this study was to investigate the associations of urinary phthalates and bisphenols at age 6 years old with body fat and cardiovascular risk factors at 6 and 10 years and with the change from 6 to 10 years.

Methods: Among 471 Dutch children, the phthalates and bisphenols urinary concentrations at 6 years and BMI, fat mass index, android fat mass, blood pressure, glucose, insulin and lipids blood concentrations at 6 and 10 years were measured.

Results: An interquartile range increase in di-n-octylphthalate (DNOP) metabolites concentrations at 6 years was associated with an increased risk of overweight at 6 and 10 years (Odds Ratio 1.44 (95% CI 1.11, 1.87), 1.43 (95% CI 1.09, 1.86), respectively). Also, higher DNOP metabolites concentrations were associated with higher fat mass index at 6 years, higher systolic blood pressure at 10 years and with a decrease in high-density lipoprotein (HDL)-cholesterol, and an increase in triglycerides concentrations from 6 to 10 years (p-values<0.05). Higher total bisphenols and bisphenol A concentrations were associated with a decrease in BMI from 6 to 10 years (p-values<0.01).

Conclusions: DNOP metabolites are associated with overweight and an adverse cardiovascular profile in childhood. Total bisphenols and bisphenol A are associated with a decrease in BMI from 6 to 10 years.

INTRODUCTION

Endocrine disrupting chemicals (EDCs), such as phthalates and bisphenols, are adverse environmental factors, which may affect childhood health.[1, 2] Phthalate metabolites are synthetic chemical esters of phthalic acid that are widely used in a variety of consumer products to impart flexibility and elasticity to plastics. Bisphenol A (BPA) is used to produce polycarbonate plastics and epoxy resins used in various products, including toys, water pipes and the lining of metal cans, and has been substituted by synthetic bisphenol analogues like bisphenol S (BPS).[3] Phthalates and bisphenols via epigenetic and endocrine mechanisms may permanently disrupt metabolic pathways contributing to an adverse cardiovascular profile .[4, 5]

High exposure to phthalate metabolites and bisphenols is increasingly reported to be associated with obesity, hypertension, insulin resistance, dyslipidemia and cardiovascular disease among adults.[6, 7] In general, fetuses and children are likely to be more vulnerable to exposure to these chemicals than adults.[8] Most previous studies have examined pregnancy as a vulnerable period rather than childhood. Results in children are mostly based on cross-sectional studies and have not revealed expected effects consistently.[9-22] Some cross-sectional studies suggested associations of higher exposure to phthalate metabolites and BPA with higher body mass index (BMI), and hip- and waist circumference.[9-11, 13] In contrast, another cross-sectional study among 845 Danish children aged 4–9 years reported that higher phthalate metabolites were negatively associated with height, weight and BMI.[19] Previous cross-sectional studies found that higher phthalate metabolites and BPA concentrations were associated with adverse childhood cardiovascular profile, such as higher blood pressure, low-grade albuminuria and insulin resistance.[14-18] However, other studies found negative or no associations between BPA exposure and childhood metabolic outcomes.[20-22]. This controversy of results from previous studies might be explained by differences in sample size, timing of collection of samples, and in the individual phthalates and bisphenols available. Also, a major literature gap is the lack of longitudinal data evaluating the association of childhood exposure to phthalate metabolites and bisphenols with body fat measures and cardiovascular risk factors.[12, 22] Assessing these associations using a longitudinal design in childhood and controlling for child´s diet and maternal exposure to phthalates metabolites and bisphenols during pregnancy will allow a better understanding of the influence of these chemicals on child´s health.

We hypothesized that increased childhood exposure to phthalate metabolites and bisphenols affect accretion of body fat and the development of elevated blood pressure and other adverse cardiovascular outcomes. We examined whether phthalate metabolites and bisphenols urinary concentrations at 6 years were associated with body fat measures and cardiovascular risk factors, including, BMI, fat mass index, android fat mass, blood pressure, and glucose, insulin, cholesterol and triglycerides concentrations at 6 and 10 years, as well as with the change in these outcomes from 6 to 10 years. Additionally, we also explored the associations of phthalate metabolites and bisphenols urinary concentrations with risks of overweight and clustering of cardiovascular risk factors in childhood.

METHODS

Study design

This study was embedded in the Generation R Study, a prospective population-based cohort study from early fetal life onward in Rotterdam, the Netherlands.[23] Phthalate metabolites and bisphenols urinary concentrations were measured among a subgroup of 775 singleton children aged 6 years. Children in this subgroup were similar to the broader Generation R cohort in terms of socio-demographic and lifestyle characteristics (**Table S1** in Supplementary Materials). We excluded children with Non-Dutch ethnicity because of potential ethnicity-specific differences in the associations.[13] The population for analysis comprises 471 Dutch children with information on phthalate metabolites and bisphenols urinary concentrations and at least one measurement of body fat and cardiovascular risk factors at 6 or 10 years (**Figure S1** in Supplementary Materials). The study was approved by the local Medical Ethics Committee of Erasmus MC (MEC 198.782/2001/31), and written informed consent was obtained from parents.

Phthalate metabolites and bisphenols urinary concentrations

Phthalate metabolites and bisphenols concentrations were measured in a spot urine sample obtained during the study visit at 6 years. As previously described, urine samples were collected between 8 a.m. and 8 p.m., stored at 4 °C and transported within 24 h of receipt to the Star Medisch Diagnostisch Centrum (STAR-MDC) laboratory to be frozen at – 20 °C. The urine specimens were shipped on dry ice in 4 ml polypropylene vials to the Wadsworth Center, New York State Department of Health, Albany, New York for analyses.[24] We grouped phthalate metabolites according to their molecular weight categories and parent compounds. BPA and BPS were grouped and used as proxy for total bisphenol exposure. Individual bisphenol and phthalate metabolites were only included in groups and assessed individually if less than 80% of the sample concentrations were below the limit of detection (LOD). We calculated the weighted molar sums for groups representing total bisphenols, low-molecular-weight (LMW) phthalates, high-molecular-weight (HMW) phthalates, and for two subgroups within HMW phthalates: di-2-ethylhexylphthalate (DEHP) and di-n-octylphthalate (DNOP) metabolites. Phthalic acid was analyzed separately as a proxy for total phthalate exposure. Phthalate metabolites and bisphenols concentrations below LOD were substituted by LOD/$\sqrt{2}$.[25] **Supplemental Table S2** shows the metabolites included in all groups, their urinary concentrations and detection rates at the age of 6 years. To account for urinary dilution, concentrations of phthalate metabolites and bisphenols were converted to microgram per gram (µg/g) creatinine (for the separate metabolites) or micromole per gram (µmol/g) creatinine (for the metabolite groups).

Body fat measures and cardiovascular risk factors

Children were invited to visit our research center at 6 and 10 years. We calculated BMI (kg/m^2) from height and weight, both measured without shoes and heavy clothing and sex- and age- adjusted z-scores of childhood BMI based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[26] BMI categories (normal weight and overweight/obesity) were obtained using the International Obesity Task Force cut-offs.[27] We measured total body fat mass by dual-energy X-ray absorptiometry (iDXA, GE140 Lunar, 2008, Madison, WI, USA, enCORE software v.12.6).[28] We divided total fat mass by height^3 at 6 years and by height^4 at 10 years in order to obtain a fat mass index uncorrelated with height after estimating the optimal adjustment by log-log regression analyses.[29, 30] We also calculated android fat mass as a percentage of total fat mass.

Blood pressure was measured at the right brachial artery four times using the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ). The mean value was calculated using the last three measurements of each participant. Non-fasting blood samples were collected to determine serum concentrations of glucose, insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides. Insulin concentrations were measured with electrochemiluminescence immunoassay (ECLIA) on the E411 module (Roche, Almere, the Netherlands). Glucose (only available at 10 years), total cholesterol, HDL-cholesterol and triglycerides concentrations were measured using the c702 module on the Cobas 8000 analyzer (Roche).

Based on previous literature, clustering of cardiovascular risk factors was defined as having three or more of the following components: systolic or diastolic blood pressure 75th percentile or above; android fat mass percentage 75th percentile or above; insulin concentration 75th percentile or above; and HDL-cholesterol 25th percentile or below or triglycerides 75th percentile or above.[31]

Covariates

Maternal age and educational level was obtained by questionnaire at enrollment. Maternal phthalate metabolites and bisphenols urinary concentrations were measured at three time points during pregnancy (median 12.9 weeks of gestation (25th-75th percentiles 12.1-14.5); median 20.4 weeks of gestation (25th-75th percentiles 19.9-20-9); median 30.2 weeks of gestation (25th-75th percentiles 29.9-30.8)) and the pregnancy-averaged concentrations were calculated.[32] Child sex and age were available from medical records. Child ethnicity was based on parental countries of birth obtained through questionnaire. Height was measured without shoes at both 6 and 10 years. The average television watching time was obtained by questionnaires at both ages. Diet quality was determined by a parent-reported food frequency questionnaire assessed at the child's mean age 8.1 years. The algorithm to score adherence to Dutch dietary guideline has been previously described and ranged from 0 to 10 on a continuous scale with higher scores reflecting better adherence to dietary guidelines.[33]

Statistical analysis

Phthalate metabolites and bisphenols concentrations were natural log-transformed to reduce variability and account for right skewedness of the distribution and standardized by the interquartile range to ease the interpretation of effect sizes. The distributions of fat mass index, insulin and triglycerides concentrations were skewed and natural log-transformed. To enable comparison of effect sizes of different outcome measures, we constructed z-scores ((observed value - mean)/ Standard deviation). We performed linear regression models to assess the associations of phthalate metabolites and bisphenols urinary concentrations at 6 years with body fat measures and cardiovascular risk factors at 6 and 10 years and with the change in these outcomes from 6 to 10 years. Non-linearity was visually assessed using a scatterplot and ruled out. Additionally, we explored, using multinominal logistic regression models, the associations of phthalate metabolites and bisphenols with risks of overweight and clustering of cardiovascular risk factors at 6 and 10 years. Due to small sample size we were not able to assess these associations with the change from 6 to 10 years. Basic models including child´s sex, age and height (only for blood pressure models) at outcome measurements. For the models with the change in the outcomes from 6 to 10 years, the corresponding change in age and height was included. Potential confounders were represented in a directed acyclic graph (DAG) (**Figure S2** in Supplementary Materials) and were included those that fulfil the graphical criteria for confounding and changed the effect estimates >10% for at least one of the outcomes. Confounder models additionally include maternal educational level and child's diet quality score and average television watching time at 6 years. For the models with the change in the outcomes from 6 to 10 years, television watching time at both ages was included since no multicollinearity issues were observed. Also, we performed a model in which we additionally adjusted for the corresponding phthalate metabolite or bisphenol pregnancy-averaged urinary concentrations. As sensitivity analyses, we additionally adjusted the models for the outcomes at 10 years and for the change in the outcomes from 6 to 10 years by the corresponding outcomes at 6 years. Based on previous literature, we tested for statistical interactions by child sex in the above analyses, but none of these were consistently significant.[10, 11] To maintain statistical power and reduce bias related to missing data on covariates, we performed multiple imputation according to the Markov Chain Monte Carlo method. The percentage of missing values for covariates ranged from 0 to 20%. Ten imputed datasets were created and no substantial differences were found between the original and imputed datasets. We present results based on pooled imputed datasets. To correct for multiple hypothesis testing, each p-value was compared with a threshold defined as 0.05 divided by the effective number of independent tests estimated based on the correlation between the exposures (p-value threshold of 0.01).[34] All statistical analyses were performed using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS IBM, Chicago, IL, USA).

RESULTS

Subject characteristics

Table 1 shows the characteristics of the study population. The mean age of the children that attended the study visits at the research center was 5.9 and 9.7 years and more than half (52.4%) were boys. Overall, 11.4% and 15.0% of children were overweight and 13.9% and 10.2% had clustering of cardiovascular risk factors at 6 and 10 years, respectively.

Table 1. Characteristics of mothers and their children[1]

	Total Group (N= 471)
Maternal characteristics	
Age, mean (SD), years	31.4 (4.2)
Education, n (%)	
Lower	6 (1.3)
Middle	161 (34.5)
Higher	300 (64.2)
Child characteristics at age 6	
Sex, n (%)	
Boys	247 (52.4)
Girls	224 (47.6)
Age at visit, mean (SD), years	5.9 (0.2)
Television watching time, n (%)	
< 2 hours	394 (89.5)
≥ 2 hours	46 (10.5)
Height, mean (SD), m	1.2 (0.0)
Body mass index, mean (SD), kg/m^2	15.9 (1.5)
BMI categories, n (%)	
Normal weight	397 (88.6)
Overweight/Obesity	51 (11.4)
Fat mass index, median (25^{th},75^{th} percentile), kg/m^3	3.1 (2.6,3.6)
Android fat mass, mean (SD), %	3.8 (0.9)
Systolic blood pressure, mean (SD), mmHg	101.7 (8.2)
Diastolic blood pressure, mean (SD), mmHg	60.0 (6.8)
Insulin, median (25^{th},75^{th} percentile), pmol/L	127.4 (66.4,191.6)
Total-cholesterol, mean (SD), mmol/L	4.2 (0.6)
HDL-cholesterol, mean (SD), mmol/L	1.3 (0.3)
Triglycerides, median (25^{th},75^{th} percentile), mmol/L	1.0 (0.8,1.4)
Clustering of cardiovascular risk factors, n (%)	
Yes	43 (13.9)
Diet quality, mean (SD), score	4.5 (1.2)

Child characteristics at age 10	
Age at visit, mean (SD), years	9.7 (0.2)
Television watching time, n (%)	
< 2 hours	299 (79.5)
≥ 2 hours	77 (20.5)
Height, mean (SD), m	1.4 (0.1)
Body mass index, mean (SD), kg/m^2	17.0 (2.3)
BMI categories, n (%)	
Normal weight	318 (85.0)
Overweight/Obesity	56 (15.0)
Fat mass index, median (25th,75th percentile), kg/m^3	2.0 (1.6,2.6)
Android fat mass, mean (SD), %	4.0 (1.2)
Systolic blood pressure, mean (SD), mmHg	102.9 (7.6)
Diastolic blood pressure, mean (SD), mmHg	58.9 (6.3)
Glucose, mean (SD), mmol/L	5.5 (0.9)
Insulin, median (25th,75th percentile), pmol/L	193.8 (114.2, 299.3)
Total-cholesterol, mean (SD), mmol/L	4.3 (0.6)
HDL-cholesterol, mean (SD), mmol/L	1.5 (0.3)
Triglycerides, median (25th,75th percentile), mmol/L	1.0 (0.7,1.3)
Clustering of cardiovascular risk factors, n (%)	
Yes	25 (10.2)

[1]Values are means (standard deviation), medians (25th,75th percentile) or numbers of subjects (valid %).

Phthalate metabolites and bisphenols urinary concentrations and body fat measures

In the confounder models, an interquartile range increase in HMW phthalates urinary concentrations was associated with higher childhood BMI at 10 years (p-value<0.05) (**Table 2**). Also, an interquartile range increase in DNOP metabolites urinary concentrations was associated with higher childhood BMI at 6 and 10 years and with an increase in childhood BMI from 6 to 10 years (p-values<0.05). The association of DNOP metabolites urinary concentrations with childhood BMI at 10 years remained significant after multiple testing correction (z-score: 0.16 (95% Confidence Interval (CI) 0.06, 0.25)). In contrast, and after multiple testing correction, an interquartile range increase in total bisphenols and BPA urinary concentrations were associated with a decrease in childhood BMI from 6 to 10 years (z-score: -0.13 (95% CI -0.22,-0.05), z-score: -0.14 (95% CI -0.23, -0.05), respectively). An interquartile range increase in HMW phthalates and DNOP metabolites urinary concentrations were also associated with higher childhood fat mass index at 6 years (p-values<0.05). However, these results did not remain significant after multiple testing correction. No associations were observed for phthalate metabolites or bisphenols urinary concentrations with android fat mass. Similar results were observed after additional adjustment for maternal phthalate metabolites and

Table 2. Associations of phthalate metabolites and bisphenols urinary concentrations with body fat measures in childhood[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores								
	Body mass index			Fat mass index			Android fat mass		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	0.02 (-0.07,0.10)	0.03 (-0.08,0.15)	0.00 (-0.08,0.08)	0.00 (-0.09,0.09)	0.02 (-0.08,0.12)	-0.00 (-0.07,0.06)	-0.02 (-0.13,0.09)	0.01 (-0.10,0.13)	-0.00 (-0.09,0.08)
LMW phthalate	0.04 (-0.04,0.12)	0.06 (-0.04,0.17)	0.01 (-0.05,0.08)	0.05 (-0.02,0.12)	0.08 (-0.01,0.17)	0.02 (-0.03,0.08)	0.04 (-0.05,0.13)	0.08 (-0.02,0.18)	0.01 (-0.06,0.08)
HMW phthalate	0.08 (-0.02,0.17)	0.14 (0.02,0.26)*	0.06 (-0.02,0.14)	0.10 (0.01,0.19)*	0.09 (-0.02,0.20)	-0.01 (-0.08,0.06)	0.05 (-0.07,0.16)	0.06 (-0.06,0.19)	0.02 (-0.08,0.10)
DEHP metabolites	0.06 (-0.05,0.16)	0.10 (-0.04,0.23)	0.04 (-0.05,0.12)	0.10 (-0.00,0.20)	0.09 (-0.02,0.21)	0.00 (-0.07,0.08)	0.05 (-0.08,0.17)	0.06 (-0.07,0.20)	0.01 (-0.09,0.11)
DNOP metabolites	0.08 (0.00,0.16)*	0.16 (0.06,0.25)* †	0.07 (0.00,0.13)*	0.08 (0.00,0.15)*	0.08 (-0.01,0.17)	-0.02 (-0.07,0.03)	0.06 (-0.03,0.15)	0.07 (-0.03,0.16)	0.00 (-0.07,0.07)
Total bisphenols	-0.01 (-0.11,0.10)	-0.13 (-0.26,0.01)	-0.13 (-0.22,-0.05)* †	-0.05 (-0.15,0.06)	-0.06 (-0.18,0.05)	-0.04 (-0.11,0.03)	-0.09 (-0.21,0.04)	-0.12 (-0.25,0.01)	-0.04 (-0.14,0.06)
BPA	0.01 (-0.10,0.12)	-0.12 (-0.26,0.02)	-0.14 (-0.23,-0.05)* †	-0.03 (-0.14,0.07)	-0.06 (-0.19,0.06)	-0.05 (-0.13,0.02)	-0.07 (-0.20,0.06)	-0.12 (-0.25,0.02)	-0.06 (-0.16,0.04)
BPS	-0.02 (-0.13,0.09)	-0.12 (-0.25,0.02)	-0.08 (-0.16,0.01)	-0.03 (-0.13,0.08)	-0.06 (-0.18,0.06)	-0.02 (-0.09,0.05)	-0.08 (-0.20,0.05)	-0.11 (-0.24,0.03)	-0.02 (-0.12,0.08)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood BMI, fat mass index and android fat mass at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age (except for sex- and age-adjusted body mass index z-scores) diet quality score and television watching time. *$p < 0.05$. † Result remained significant after multiple testing correction.

bisphenols pregnancy-averaged urinary concentrations (**Table S3** in Supplementary Materials). Sensitivity analyses showed that, after adjustment for the corresponding outcome at 6 years, results were largely similar, although the effect estimates were slightly attenuated, especially for the associations of HMW phthalates and DNOP metabolites urinary concentrations with childhood BMI at 10 years (**Table S4** in Supplementary Materials). Results from the basic models are given in **Supplemental Table S5**. **Figure 1** shows that after adjustment for confounding, an interquartile range increase in HMW phthalates and DNOP metabolites urinary concentrations were associated with higher risk of childhood overweight at both ages (p-values<0.05). The associations of DNOP metabolites urinary concentrations with risk of childhood overweight at 6 and 10 years remained significant after multiple testing correction (Odds Ratio 1.44 (95% CI 1.11, 1.87), 1.43 (95% CI 1.09, 1.86), respectively). Results from the basic models are given in **Supplemental Figure S3**.

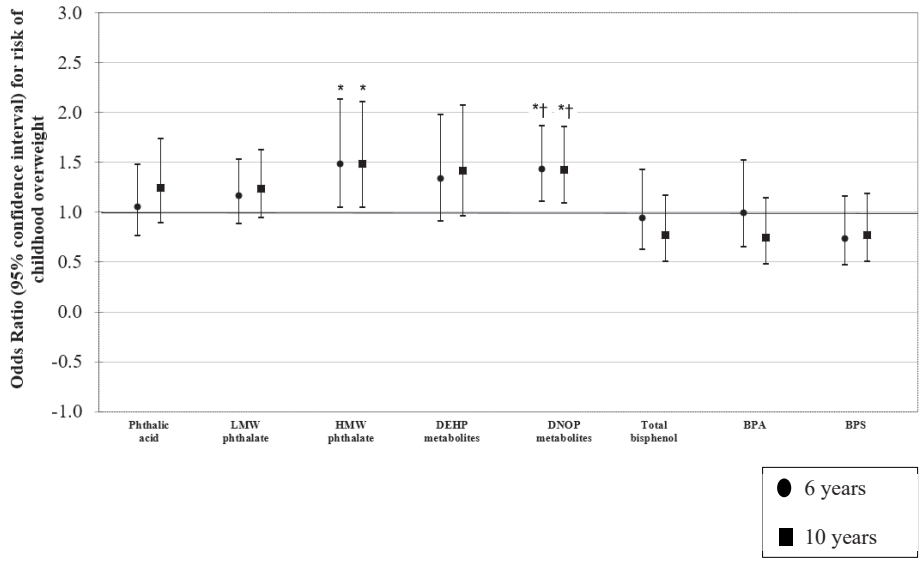

Figure 1. Associations of phthalate metabolites and bisphenols urinary concentrations with risk of overweight in childhood[1]

[1]Values are odds ratios (95% Confidence Intervals) on a logarithmic scale and represent the risk of childhood overweight and obesity at 6 and 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Models are adjusted for maternal educational level and child diet quality score and television watching time. *p-value < 0.05. † Result remained significant after multiple testing correction.

Phthalate metabolites and bisphenols urinary concentrations and cardiovascular risk factors

An interquartile range increase in phthalic acid urinary concentrations was associated with an increase in childhood systolic blood pressure from 6 to 10 years (p-value<0.05) (**Table 3**). Also, an interquartile range increase in HMW phthalates and DNOP metabolites urinary

Table 3. Associations of phthalate metabolites and bisphenols urinary concentrations with blood pressure in childhood[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores					
	Systolic blood pressure			Diastolic blood pressure		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.03 (-0.14,0.07)	0.07 (-0.05,0.18)	0.14 (0.03,0.25)*	0.02 (-0.09,0.12)	0.02 (-0.10,0.14)	0.09 (-0.05,0.23)
LMW phthalate	-0.00 (-0.09,0.09)	0.03 (-0.06,0.13)	0.04 (-0.06,0.13)	0.04 (-0.05,0.13)	0.01 (-0.10,0.11)	0.00 (-0.12,0.12)
HMW phthalate	0.08 (-0.03,0.20)	0.13 (0.01,0.25)*	0.07 (-0.05,0.19)	0.12 (0.01,0.23)*	0.09 (-0.04,0.22)	0.04 (-0.11,0.18)
DEHP metabolites	0.05 (-0.07,0.18)	0.10 (-0.03,0.23)	0.08 (-0.05,0.21)	0.10 (-0.03,0.22)	0.05 (-0.09,0.18)	0.03 (-0.14,0.19)
DNOP metabolites	0.06 (-0.03,0.15)	0.10 (0.00,0.19)*	0.06 (-0.03,0.16)	0.08 (-0.01,0.17)	0.09 (-0.01,0.19)	0.04 (-0.08,0.16)
Total bisphenols	-0.01 (-0.13,0.12)	-0.02 (-0.15,0.11)	-0.03 (-0.17,0.10)	-0.13 (-0.25,-0.00)*	0.01 (-0.13,0.15)	0.18 (0.02,0.35)*
BPA	-0.02 (-0.16,0.11)	-0.03 (-0.16,0.11)	-0.02 (-0.16,0.12)	-0.13 (-0.26,-0.00)*	0.00 (-0.14,0.15)	0.18 (0.01,0.35)*
BPS	0.10 (-0.03,0.23)	0.08 (-0.06,0.21)	-0.02 (-0.15,0.12)	-0.03 (-0.16,0.10)	0.08 (-0.06,0.22)	0.14 (-0.02,0.31)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood blood pressure at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, height, diet quality score and television watching time. *p-value < 0.05.

concentrations were associated with higher childhood systolic blood pressure at 10 years (p-values<0.05). An interquartile range increase in HMW phthalates urinary concentrations was associated with higher diastolic blood pressure at 6 years while an interquartile range increase in total bisphenols and BPA urinary concentrations were associated with lower diastolic blood pressure at 6 years (p-values<0.05). Also, an interquartile range increase in total bisphenols and BPA urinary concentrations were associated with an increase in childhood diastolic blood pressure from 6 to 10 years (p-values<0.05). However, all these associations did not remain significant after multiple testing correction. An interquartile range increase in LMW phthalates and DEHP metabolites urinary concentrations were associated with lower HDL-cholesterol and higher triglycerides concentrations at 10 years, respectively (p-values<0.05) (**Table 4**). Also, an interquartile range increase in DNOP metabolites urinary concentrations was associated with a decrease in HDL-cholesterol and an increase in triglycerides from 6 to 10 years (p-values<0.05). In contrast, an interquartile range increase in total bisphenols and BPA urinary concentrations were associated with lower insulin concentrations at 10 years and with higher HDL-cholesterol at 6 years (p-values<0.05). However, only the association of DNOP metabolites urinary concentrations with a decrease in HDL-cholesterol

from 6 to 10 years remained significant after multiple testing correction (z-score: -0.17 (95% CI -0.28,-0.06)). Phthalate metabolites and bisphenols urinary concentrations were not associated with glucose concentrations at 10 years (**Table S6** in Supplementary Materials). Similar results were observed after additional adjustment for maternal phthalate metabolites and bisphenols pregnancy-averaged urinary concentrations (**Tables S6, S7** and **S8** in Supplementary Materials). Sensitivity analyses showed that, after adjustment for the corresponding outcome at 6 years, results were similar but the effect estimates were attenuated. In contrast, the association of DNOP metabolites urinary concentrations with lower HDL-cholesterol at 10 years reached statistical significance even after multiple testing correction (z-score: -0.14 (95% CI -0.24,-0.04)) (**Tables S9** and **S10** in Supplementary Materials). Results from the basic models are given in **Tables S6**, **S11** and **S12** in Supplementary Materials. **Figure 2** shows that after adjustment for confounding, an interquartile range increase in LMW phthalates was associated with a higher risk of clustering of cardiovascular risk factors at 10 years while an interquartile range increase in HMW phthalates was associated with a lower risk of clustering of cardiovascular risk factors at 6 years (p-values<0.05). These associations did not remain significant after multiple testing correction. Bisphenols urinary concentrations were not associated with clustering of cardiovascular risk factors. Results from the basic models are given in **Supplemental Figure S4**.

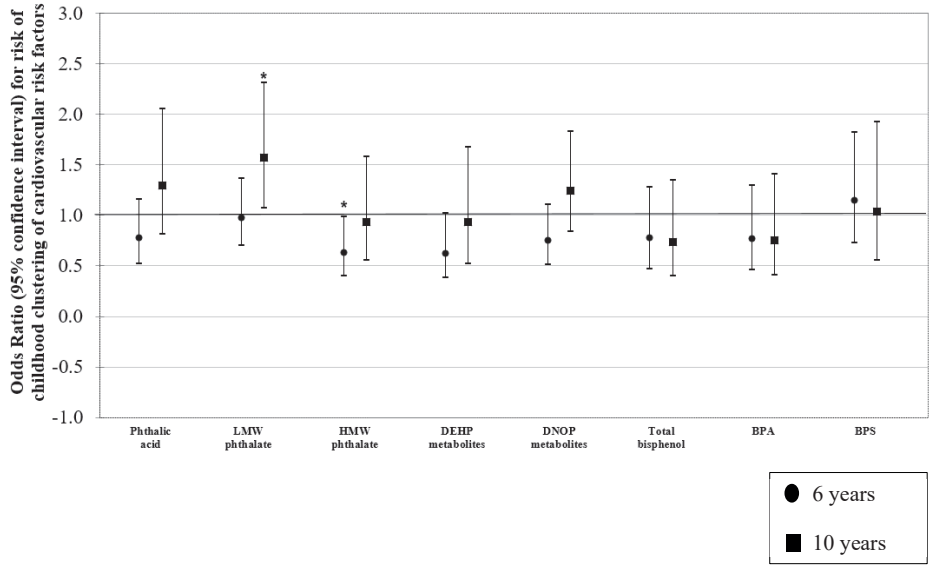

Figure 2. Associations of phthalate metabolites and bisphenols urinary concentrations with risk of clustering of cardiovascular risk factors in childhood[1]

[1]Values are odds ratios (95% Confidence Intervals) on a logarithmic scale and represent the risk of clustering of cardiovascular risk factors at 6 and 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Models are adjusted for maternal educational level and child sex, age, diet quality score and television watching time. *p-value < 0.05.

Table 4. Associations of phthalate metabolites and bisphenols urinary concentrations with insulin and lipids profile in childhood[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores											
	Insulin			Total Cholesterol			HDL Cholesterol			Triglycerides		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.06 (-0.18,0.06)	-0.03 (-0.16,0.11)	0.04 (-0.17,0.24)	0.04 (-0.08,0.16)	0.09 (-0.05,0.22)	-0.03 (-0.14,0.08)	0.06 (-0.05,0.18)	-0.05 (-0.18,0.08)	-0.07 (-0.20,0.05)	0.07 (-0.05,0.19)	0.06 (-0.07,0.19)	-0.02 (-0.21,0.17)
LMW phthalate	-0.04 (-0.14,0.07)	0.04 (-0.08,0.16)	0.05 (-0.13,0.23)	0.06 (-0.05,0.16)	0.09 (-0.03,0.20)	-0.03 (-0.13,0.06)	0.00 (-0.10,0.10)	-0.12 (-0.23,-0.00)*	-0.09 (-0.20,0.01)	0.08 (-0.03,0.18)	0.09 (-0.03,0.20)	-0.01 (-0.18,0.15)
HMW phthalate	-0.07 (-0.20,0.06)	0.01 (-0.14,0.16)	0.11 (-0.11,0.33)	0.02 (-0.11,0.15)	0.03 (-0.11,0.17)	-0.00 (-0.12,0.12)	0.07 (-0.05,0.20)	0.03 (-0.11,0.17)	-0.09 (-0.22,0.04)	-0.02 (-0.15,0.11)	0.14 (-0.01,0.28)	0.16 (-0.04,0.37)
DEHP metabolites	-0.08 (-0.22,0.06)	0.04 (-0.12,0.20)	0.15 (-0.10,0.40)	0.03 (-0.12,0.17)	0.07 (-0.09,0.22)	0.02 (-0.11,0.15)	0.10 (-0.04,0.23)	0.07 (-0.08,0.23)	-0.03 (-0.18,0.11)	0.03 (-0.12,0.17)	0.19 (0.03,0.34)*	0.14 (-0.08,0.37)
DNOP metabolites	-0.04 (-0.14,0.07)	-0.00 (-0.13,0.12)	0.07 (-0.12,0.26)	0.01 (-0.09,0.12)	-0.03 (-0.15,0.10)	0.00 (-0.10,0.10)	0.01 (-0.09,0.11)	-0.08 (-0.20,0.04)	-0.17 (-0.28,-0.06)*†	-0.09 (-0.20,0.01)	0.06 (-0.06,0.18)	0.19 (0.02,0.37)*
Total bisphenols	-0.03 (-0.18,0.13)	-0.17 (-0.33,-0.01)*	-0.17 (-0.43,0.09)	0.10 (-0.05,0.25)	-0.01 (-0.16,0.15)	-0.12 (-0.25,0.02)	0.17 (0.02,0.32)*	0.01 (-0.15,0.16)	-0.07 (-0.22,0.08)	0.01 (-0.14,0.17)	-0.06 (-0.21,0.10)	-0.13 (-0.37,0.10)
BPA	-0.03 (-0.19,0.13)	-0.19 (-0.36,-0.03)*	-0.19 (-0.46,0.07)	0.07 (-0.09,0.23)	-0.04 (-0.20,0.12)	-0.12 (-0.26,0.02)	0.20 (0.04,0.35)*	0.01 (-0.15,0.17)	-0.08 (-0.24,0.07)	0.02 (-0.14,0.18)	-0.02 (-0.18,0.14)	-0.09 (-0.33,0.16)
BPS	-0.02 (-0.17,0.13)	0.06 (-0.10,0.22)	0.07 (-0.17,0.31)	0.14 (-0.01,0.29)	0.04 (-0.12,0.19)	-0.05 (-0.17,0.08)	-0.02 (-0.16,0.13)	-0.04 (-0.19,0.11)	0.04 (-0.10,0.18)	0.10 (-0.06,0.25)	-0.10 (-0.25,0.06)	-0.15 (-0.37,0.08)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood insulin and lipids profile at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, diet quality score and television watching time. *p-value < 0.05. † Result remained significant after multiple testing correction.

DISCUSSION

In a population-based study, we observed that higher DNOP metabolites urinary concentrations were associated with an increased risk of overweight and obesity and with lower HDL-cholesterol and tended to be associated with higher systolic blood pressure and higher triglycerides in school-age children. Higher total bisphenols and BPA urinary concentrations were associated with lower BMI and tended to be associated with higher diastolic blood pressure and lower insulin in school-age children.

Interpretation of main findings

As a result of the widespread use of phthalate metabolites and bisphenols-related products, children can be exposed to these potential harmful chemicals through different pathways, such as ingestion, inhalation and dermal contact.[11] Phthalates and bisphenols may interfere with endocrine processes resulting in a deviation from the normal homeostatic control that may lead to an adverse cardiovascular profile.[1] We hypothesized that increased exposure to phthalate metabolites and bisphenols affect body fat and cardiovascular development already in childhood.

A previous narrative review reported positive associations of exposure to phthalate metabolites with childhood BMI, subscapular skinfold thickness, and hip- and waist circumference in five studies and the associations were mostly observed among boys.[3] However, a recent meta-analysis of 29 studies in children and adults has reported inconsistencies in results from published literature on the association between the exposure to phthalates and adiposity.[35] In the current study, we observed that higher HMW phthalate concentrations, specifically DNOP metabolites, at 6 years were associated with higher BMI and an increased risk of overweight and obesity in school-age children. Contrarily to previous studies and surprisingly due to estrogenicity of bisphenols and antiandrogenicity of some phthalates, we did not observe a statistical interaction by child´s sex. However, we cannot exclude the possibility that our results might have been underpowered to detect differences by sex due to small sample size. Most studies of bisphenols were only focused on BPA and reported that, in childhood, higher BPA concentrations were associated with increased BMI, hip- and waist circumference and body fat.[9, 21] However, we observed that higher total bisphenols and BPA urinary concentrations were associated with a decrease in BMI from 6 to 10 years but not with fat mass index or android fat mass. Based on previous studies, we did not hypothesize this association beforehand. Although we cannot disregard the possibility of a true association, it might be due to residual confounding.

An accumulating body of evidence suggests that phthalate metabolites and bisphenols exposure may contribute to acute and chronic cardiovascular risks, altered blood pressure, and atherosclerosis, as well as low grade inflammation, diabetes, insulin resistance and hyperlipidemia later in life.[6, 7] The associations of phthalate metabolites and bisphenols exposure

with cardiovascular outcomes have also been explored during childhood.[14-18, 20, 22] Previous studies reported that childhood exposure to phthalate metabolites and bisphenols is associated with an adverse metabolic profile, such as increased blood pressure and low-grade albuminuria.[14-16, 20] However, a study among 2,555 US children aged 6–19 years did not report an association of phthalate metabolites with triglycerides and HDL-cholesterol concentrations.[14] Also, other studies did not report associations of BPA exposure with childhood metabolic outcomes, including glucose, insulin resistance and blood lipids from mid-childhood until adolescence.[21, 22] In the present study and similarly to previous studies, we observed that higher phthalic acid and HMW phthalates, specifically DNOP metabolites, tended to be associated with higher childhood systolic blood pressure. We also observed that higher LMW phthalates, DEHP metabolites and DNOP metabolites tended to be associated with lower HDL-cholesterol and higher triglycerides concentrations in school-age children. On the other hand, we observed that higher total bisphenols and BPA urinary concentrations tended to be associated with an increase in diastolic blood pressure from 6 to 10 years and with lower insulin at 10 years. The positive association of total bisphenols and BPA with diastolic blood pressure from 6 to 10 years should be interpreted with caution since the effect estimates attenuated after additional adjustment for the outcome at 6 years and a negative association was observed at 6 years.

The potential mechanisms underlying the associations of phthalate metabolites with overweight and an adverse cardiovascular profile might include the activation of peroxisome proliferator-activated receptors (PPARs) and imbalance of steroid and thyroid hormones.[36-38] Activation of PPARs can increase lipid accumulation and release adipocyte-related hormones leading to higher susceptibility for the development of obesity.[36] Likewise, the perturbation of the steroid and thyroid hormones system that are critical for the maintenance of basal metabolism may also have obesogenic effects.[37, 38] Similar mechanisms have been found for bisphenols.[36, 37] However, this is not in line with our results, which showed an association of bisphenols with lower BMI in children.

Altogether, our results suggest that DNOP metabolites and bisphenols exposure may affect childhood BMI. The associations of phthalate metabolites and bisphenols with cardiovascular risk factors, except for DNOP metabolites and HDL-cholesterol from 6 to 10 years, were no longer significant after multiple testing correction and thus we cannot exclude the possibility of results being chance findings. The observed effect estimates might be small on an individual level, but can be important on a population-based level since children are widely exposed to these EDCs and overweight and obesity and adverse cardiovascular risk factors tend to track into poorer cardiovascular health later in life. Due to the observational design of this study, we cannot draw conclusions about causality. Further studies are needed to replicate these findings and investigate potential mechanisms.

Strengths and limitations

The major strengths of this study were the availability of urinary measurements of diverse phthalate metabolites and BPS and the detailed data available on childhood body fat measures and cardiovascular risk factors. Also, contrarily to most previous studies that were embedded in a cross-sectional design and thus can be affected by reverse causality, we were able to address the associations of exposure to phthalate metabolites and bisphenols at 6 years with body fat and cardiovascular risk factors at 10 years. This study also has limitations. This study was conducted in a low-risk small sample, which might have resulted in insufficient power to detect associations, especially after conducting multiple testing correction. No substantial differences in terms of socio-demographic and lifestyle characteristics were observed between children in this subgroup and in the broader cohort and thus, although it cannot be excluded, selection bias seems unlikely. In our study, we relied on a single-spot urinary measurement of phthalate metabolites and bisphenols as an estimate of exposure. Both phthalate metabolites and bisphenols have short biological half-lives [39, 40], although it has been suggested that a single urine sample for phthalate concentrations reasonably reflects exposure for up to 3 months.[41] Thus, measurement error may have led to underestimation of the effect estimates, especially for bisphenols. Moreover, the use of non-fasting blood samples of childhood glucose, insulin and lipids profile may also have led to underestimation of the observed associations. However, previous studies have shown that semi-fasted insulin resistance is moderately correlated with fasting values [42] and that non-fasting blood lipids levels can accurately predict increased risks of cardiovascular events later in life.[43] We collected information on many potential confounding variables, but residual confounding due to unmeasured lifestyle variables might still be an issue. Previous evidence supports a link between early puberty and adiposity.[44] We do not have information on pubertal development. Future studies should assess these associations considering pubertal status of the children. The current study was focused on phthalate and bisphenol urinary concentrations. Other EDCs, such as pesticides, might be related with adiposity outcomes in children.[45] These associations should be explored in future studies.

CONCLUSION

Our study suggests that adiposity in school-aged children may be influenced by phthalate metabolites and bisphenols exposure, specifically by DNOP metabolites and BPA. DNOP metabolites seem also to be associated with an adverse cardiovascular profile in childhood. Further studies are needed, both to replicate our findings and to explore the potential mechanisms involved.

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SUPPLEMENTARY MATERIALS

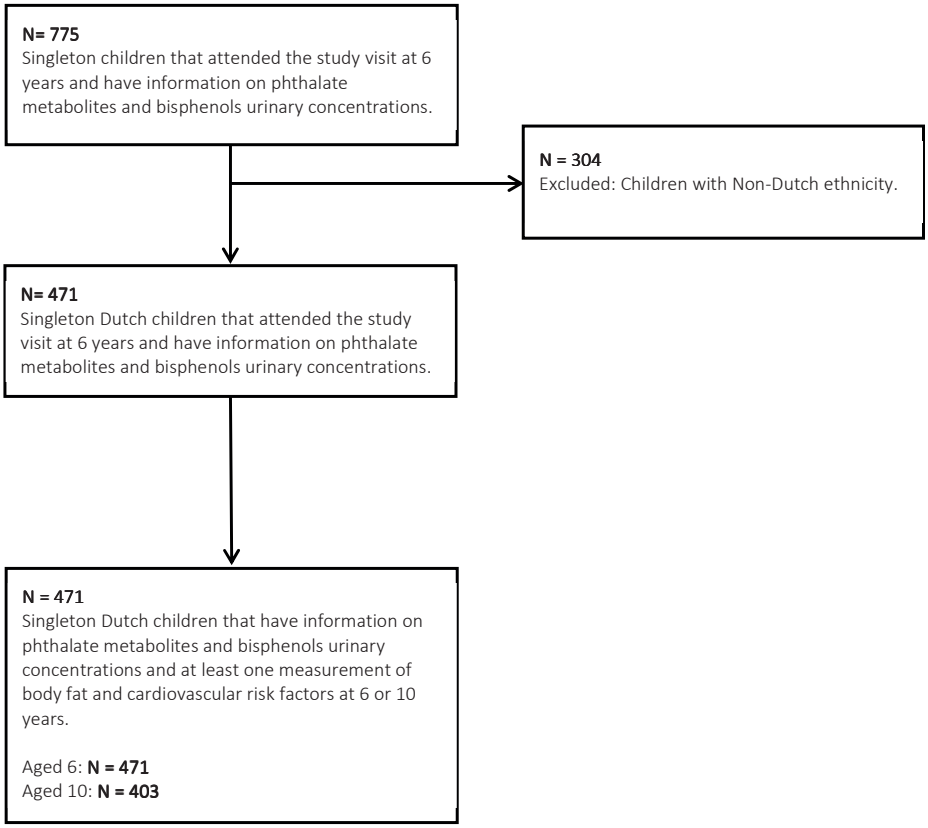


Figure S1. Flowchart of study participants

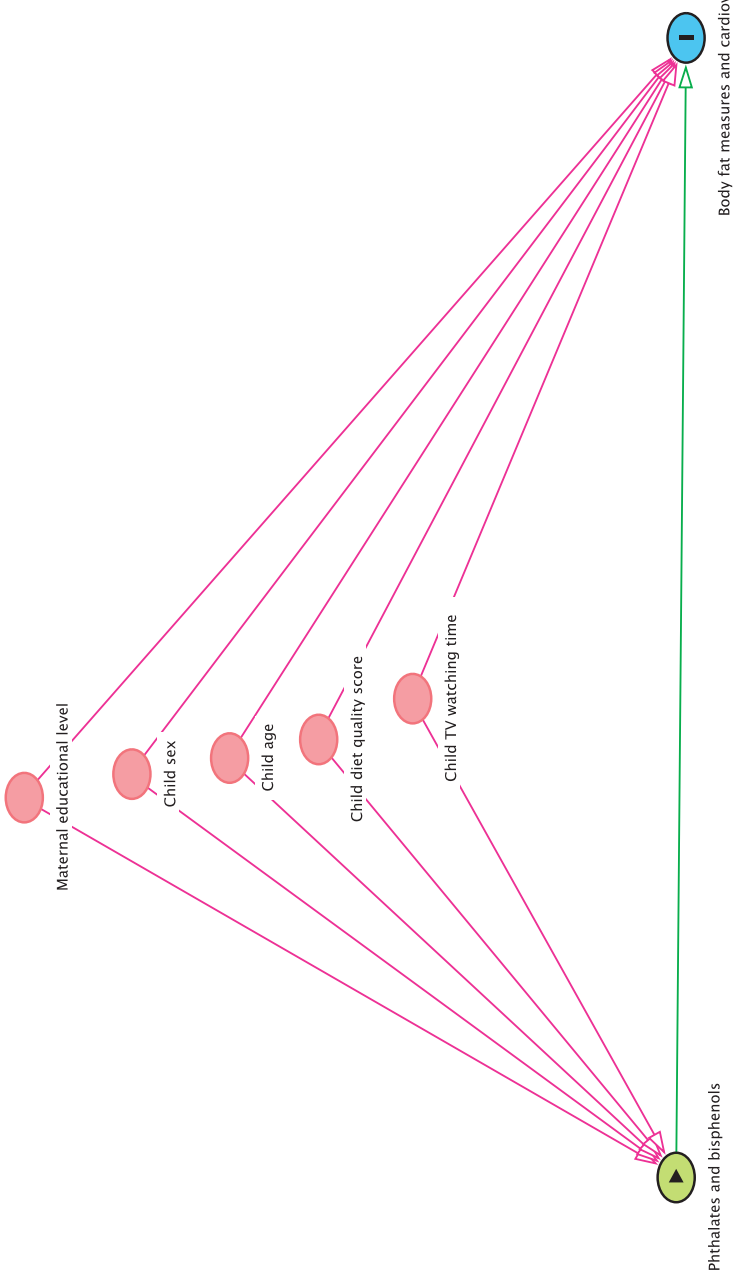


Figure S2. Directed Acyclic Graph showing the hypothesized relationship between phthalate metabolites and bisphenols urinary concentrations, childhood body fat measures, cardiovascular risk factors and the covariates

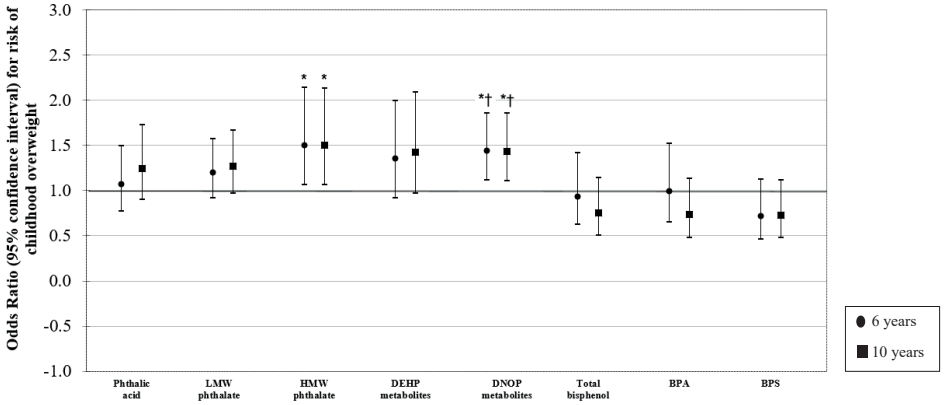


Figure S3. Associations of phthalate metabolites and bisphenols urinary concentrations with risk of overweight in childhood, basic models[1]

[1]Values are odds ratios (95% Confidence Intervals) on a logarithmic scale and represent the risk of childhood overweight and obesity at 6 and 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Models are unadjusted. *p-value < 0.05. † Result remained significant after multiple testing correction.

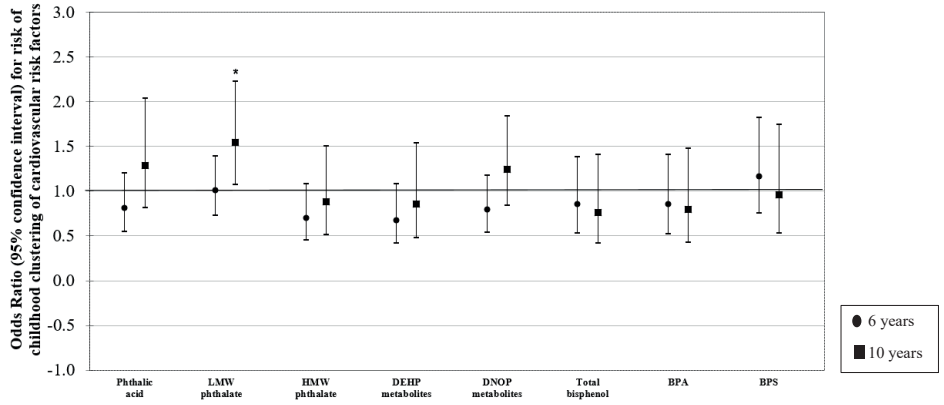


Figure S4. Associations of phthalate metabolites and bisphenols urinary concentrations with risk of clustering of cardiovascular risk factors in childhood, basic models[1]

[1]Values are odds ratios (95% Confidence Intervals) on a logarithmic scale and represent the risk of clustering of cardiovascular risk factors at 6 and 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Models are adjusted for child sex and age. *p-value < 0.05.

Table S1. Comparison of characteristics between the subgroup with phthalate metabolites and bisphenols urinary concentrations and the broader Generation R cohort[1]

	Subgroup (N=775)	Generation R (N=6,523)
Maternal characteristics		
Age, mean (SD), years	30.7 (4.6)	30.5 (5.2)
Education, n (%)		
Lower	42 (5.6)	566 (9.5)
Middle	300 (40.2)	2,601 (43.9)
Higher	405 (54.2)	2,762 (46.6)
Child characteristics at age 6		
Sex, n (%)		
Boys	398 (51.4)	3,273 (50.2)
Girls	377 (48.6)	3,250 (49.8)
Age at visit, mean (SD), years	5.9 (0.2)	6.2 (0.5)
Television watching time, n (%)		
< 2 hours	583 (83.5)	4,071 (80.6)
≥ 2 hours	115 (16.5)	983 (19.4)
Height, mean (SD), m	1.2 (0.0)	1.2 (0.1)
Body mass index, mean (SD), kg/m^2	16.1 (1.6)	16.2 (1.9)
BMI categories, n (%)		
Normal weight	614 (83.9)	5,031 (81.4)
Overweight/Obesity	118 (16.1)	1,149 (18.6)
Fat mass index, median (25^{th},75^{th} percentile), kg/m^3	3.1 (2.6,3.8)	3.1 (2.7,3.8)
Android fat mass, mean (SD), %	3.8 (0.9)	3.8 (1.0)
Systolic blood pressure, mean (SD), mmHg	102.2 (8.4)	102.7 (8.2)
Diastolic blood pressure, mean (SD), mmHg	60.2 (6.8)	60.7 (6.8)
Insulin, median (25^{th},75^{th} percentile), pmol/L	130.0 (66.2,199.6)	112.3 (62.6,187.0)
Total-cholesterol, mean (SD),mmol/L	4.2 (0.6)	4.2 (0.6)
HDL-cholesterol, mean (SD), mmol/L	1.4 (0.3)	1.4 (0.3)
Triglycerides, median (25^{th},75^{th} percentile), mmol/L	1.0 (0.7,1.3)	0.9 (0.7,1.3)

[1]Values are means (standard deviation), medians (25^{th},75^{th} percentile) or numbers of subjects (valid %).

Table S2. Phthalate metabolites and bisphenols urinary concentrations at 6 years[1]

	LOD	Median (25th,75th percentile)	% below LOD
Phthalic Acid	6.68	165.8 (102.6,254.9)	0
Low molecular weight phthalate		225.0 (114.7,430.4)	
Monomethylphthalate	0.33	17.5 (3.3,32.0)	17.3
Monoethylphthalate	0.31	68.0 (31.6,158.6)	0
Mono-isobutylphthalate	0.40	58.5 (33.3,105.3)	0.5
Mono-n-butylphthalate	0.63	29.7 (15.2,55.6)	2.1
High molecular weight phthalate		92.7 (50.7,166.6)	
Monobenzylphthalate	0.59	2.8 (0.4,12.3)	40.4
DNOP			
Mono(3-carboxypropyl)phthalate	0.03	5.2 (2.8,9.4)	0
DEHP		79.1 (44.7,130.3)	
Mono-(2-ethyl-5-carboxypentyl)phthalate	0.94	27.8 (14.5,50.2)	0
Mono-[(2-carboxymethyl)hexyl]phthalate	0.13	12.3 (7.2,20.1)	0
Mono-(2-ethyl-5-hydroxyhexyl)phthalate	0.27	22.7 (12.8,37.2)	0
Mono-(2-ethyl-5oxohexyl)phthalate	0.14	12.9 (6.8,21.5)	0
Bisphenols		2.8 (0.9,5.6)	
Bisphenol A	0.66	2.5 (0.5,5.0)	26.2
Bisphenol S	0.20	0.13 (0.13,0.32)	74.8

[1]Absolute urinary concentrations of grouped exposures (in nmol/L urine) and individual exposures (in nmol/L urine) with concentrations below the limit of detection imputed as limit of detection/square root of 2. The limit of detection is expressed in nmol/L urine. LOD, limit of detection; DEHP, di-2-ethylhexylphthalate; DNOP, di-n-octylphthalate.

Table S3. Associations of phthalate metabolites and bisphenols urinary concentrations with body fat measures in childhood, prenatal exposure model[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores								
	Body mass index			Fat mass index			Android fat mass		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	0.02 (-0.06,0.11)	0.04 (-0.07,0.16)	0.01 (-0.07,0.08)	0.01 (-0.08,0.09)	0.03 (-0.07,0.13)	0.00 (-0.06,0.07)	-0.01 (-0.12,0.09)	0.02 (-0.09,0.14)	0.00 (-0.09,0.09)
LMW phthalate	0.04 (-0.03,0.12)	0.07 (-0.03,0.17)	0.02 (-0.05,0.09)	0.05 (-0.02,0.13)	0.08 (-0.01,0.17)	0.03 (-0.03,0.08)	0.05 (-0.04,0.14)	0.09 (-0.01,0.19)	0.01 (-0.07,0.08)
HMW phthalate	0.08 (-0.02,0.17)	0.14 (0.02,0.26)*	0.06 (-0.02,0.14)	0.11 (0.02,0.20)*	0.10 (-0.01,0.21)	-0.01 (-0.08,0.06)	0.05 (-0.06,0.16)	0.06 (-0.06,0.19)	0.01 (-0.08,0.10)
DEHP metabolites	0.06 (-0.04,0.16)	0.10 (-0.03,0.23)	0.04 (-0.05,0.13)	0.10 (0.00,0.20)*	0.10 (-0.02,0.22)	0.00 (-0.07,0.07)	0.05 (-0.07,0.18)	0.06 (-0.07,0.20)	0.01 (-0.09,0.11)
DNOP metabolites	0.08 (0.01,0.16)*	0.16 (0.07,0.26)*†	0.08 (0.01,0.14)*	0.08 (0.00,0.15)*	0.08 (-0.00,0.17)	-0.01 (-0.07,0.04)	0.06 (-0.03,0.15)	0.07 (-0.03,0.17)	0.01 (-0.07,0.08)
Total bisphenols	0.00 (-0.10,0.11)	-0.10 (-0.23,0.03)	-0.13 (-0.22,-0.04)*†	-0.02 (-0.12,0.08)	-0.04 (-0.15,0.08)	-0.04 (-0.11,0.03)	-0.07 (-0.20,0.05)	-0.09 (-0.22,0.04)	-0.04 (-0.13,0.06)
BPA	0.02 (-0.09,0.13)	-0.09 (-0.23,0.05)	-0.14 (-0.23,-0.05)*†	-0.01 (-0.11,0.10)	-0.04 (-0.16,0.09)	-0.05 (-0.13,0.02)	-0.05 (-0.18,0.09)	-0.09 (-0.22,0.05)	-0.06 (-0.16,0.04)
BPS	-0.04 (-0.14,0.07)	-0.12 (-0.25,0.02)	-0.07 (-0.16,0.02)	-0.04 (-0.14,0.07)	-0.06 (-0.18,0.06)	-0.02 (-0.09,0.06)	-0.09 (-0.22,0.04)	-0.10 (-0.23,0.03)	-0.01 (-0.11,0.09)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood BMI, fat mass index and android fat mass at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and exposure to phthalate metabolites and bisphenols during pregnancy (natural log-transformed pregnancy-averaged urinary concentrations in µmol/g creatinine) and child sex, age (except for sex- and age- adjusted body mass index z-scores), diet quality score and television watching time. *p-value < 0.05. † Result remained significant after multiple testing correction.

Table S4. Associations of phthalate metabolites and bisphenols urinary concentrations with body fat measures in childhood, sensitivity analyses[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores					
	Body mass index		Fat mass index		Android fat mass	
	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years
Phthalic acid	0.01 (-0.07,0.08)	0.00 (-0.07,0.08)	0.00 (-0.06,0.07)	0.00 (-0.06,0.06)	0.01 (-0.07,0.09)	0.01 (-0.07,0.08)
LMW phthalate	0.02 (-0.04,0.09)	0.02 (-0.05,0.09)	0.03 (-0.02,0.08)	0.03 (-0.03,0.08)	0.03 (-0.04,0.10)	0.03 (-0.04,0.10)
HMW phthalate	0.07 (-0.01,0.15)	0.07 (-0.01,0.15)	0.00 (-0.06,0.07)	0.00 (-0.06,0.07)	0.03 (-0.06,0.11)	0.03 (-0.06,0.11)
DEHP metabolites	0.05 (-0.04,0.13)	0.04 (-0.05,0.13)	0.02 (-0.06,0.07)	0.02 (-0.06,0.09)	0.03 (-0.06,0.12)	0.03 (-0.07,0.12)
DNOP metabolites	0.08 (0.01,0.14)*	0.08 (0.02,0.14)*	-0.01 (-0.06,0.04)	-0.00 (-0.06,0.05)	0.02 (-0.05,0.09)	0.02 (-0.05,0.09)
Total bisphenols	-0.13 (-0.22,-0.05)*†	-0.13 (-0.22,-0.05)*†	-0.05 (-0.12,0.03)	-0.05 (-0.12,0.02)	-0.06 (-0.15,0.03)	-0.06 (-0.15,0.03)
BPA	-0.14 (-0.23,-0.05)*†	-0.14 (-0.23,-0.05)*†	-0.06 (-0.13,0.02)	-0.06 (-0.13,0.02)	-0.07 (-0.17,0.02)	-0.07 (-0.17,0.02)
BPS	-0.09 (-0.17,0.00)	-0.08 (-0.17,0.01)	-0.04 (-0.11,0.03)	-0.03 (-0.10,0.04)	-0.04 (-0.13,0.05)	-0.04 (-0.13,0.05)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood BMI, fat mass index and android fat mass at 10 years and the change in the outcomes in z-scores from 6 to 10 years for an inter-quartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age (except for sex- and age- adjusted body mass index z-scores), diet quality score, television watching time and additionally for the corresponding outcome at 6 years. *p-value < 0.05. †Result remained significant after multiple testing correction.

Table S5. Associations of phthalate metabolites and bisphenols urinary concentrations with body fat measures in childhood, basic models[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores								
	Body mass index			Fat mass index			Android fat mass		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	0.02 (-0.07,0.10)	0.04 (-0.08,0.15)	0.01 (-0.07,0.08)	0.01 (-0.07,0.10)	0.03 (-0.08,0.13)	0.00 (-0.06,0.07)	-0.01 (-0.10,0.09)	0.02 (-0.09,0.12)	0.00 (-0.08,0.09)
LMW phthalate	0.04 (-0.03,0.12)	0.08 (-0.02,0.18)	0.03 (-0.04,0.10)	0.07 (-0.00,0.15)	0.09 (0.00,0.18)*	0.03 (-0.03,0.08)	0.05 (-0.03,0.13)	0.09 (-0.00,0.18)	0.02 (-0.05,0.10)
HMW phthalate	0.08 (-0.01,0.17)	0.14 (0.01,0.26)*	0.06 (-0.03,0.14)	0.11 (0.02,0.20)*	0.10 (-0.02,0.21)	-0.01 (-0.08,0.05)	0.05 (-0.05,0.15)	0.06 (-0.05,0.17)	0.01 (-0.08,0.10)
DEHP metabolites	0.06 (-0.04,0.16)	0.09 (-0.04,0.23)	0.03 (-0.06,0.12)	0.10 (0.00,0.20)*	0.09 (-0.03,0.21)	0.00 (-0.07,0.07)	0.05 (-0.06,0.16)	0.06 (-0.06,0.17)	0.01 (-0.09,0.10)
DNOP metabolites	0.08 (0.01,0.16)*	0.16 (0.06,0.26)*†	0.07 (0.00,0.13)*	0.09 (0.02,0.16)*	0.09 (-0.00,0.17)	-0.02 (-0.07,0.03)	0.06 (-0.02,0.14)	0.06 (-0.03,0.15)	0.00 (-0.07,0.08)
Total bisphenols	-0.00 (-0.11,0.10)	-0.12 (-0.26,0.02)	-0.13 (-0.22,-0.04)*†	-0.05 (-0.15,0.06)	-0.06 (-0.18,0.06)	-0.04 (-0.11,0.04)	-0.08 (-0.19,0.03)	-0.11 (-0.22,0.01)	-0.04 (-0.13,0.06)
BPA	0.01 (-0.10,0.12)	-0.12 (-0.26,0.02)	-0.14 (-0.23,-0.05)*†	-0.03 (-0.14,0.08)	-0.06 (-0.19,0.06)	-0.05 (-0.12,0.03)	-0.06 (-0.17,0.06)	-0.10 (-0.22,0.02)	-0.06 (-0.16,0.05)
BPS	-0.02 (-0.13,0.08)	-0.13 (-0.27,0.00)	-0.09 (-0.18,-0.00)*	-0.04 (-0.15,0.06)	-0.08 (-0.20,0.04)	-0.03 (-0.10,0.04)	-0.08 (-0.19,0.03)	-0.11 (-0.23,0.00)	-0.03 (-0.13,0.07)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood BMI, fat mass index and android fat mass at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Basic models include child´s sex and age (except for sex- and age- adjusted body mass index z-scores). *p < 0.05. † Result remained significant after multiple testing correction.

Table S6. Associations of phthalate metabolites and bisphenols urinary concentrations with glucose in childhood1

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores		
	Basic model	Confounder model	Prenatal exposure model
	10 years	10 years	10 years
Phthalic acid	-0.04 (-0.17,0.10)	-0.05 (-0.19,0.09)	-0.05 (-0.19,0.09)
LMW phthalate	-0.07 (-0.19,0.05)	-0.07 (-0.19,0.05)	-0.07 (-0.20,0.05)
HMW phthalate	-0.06 (-0.21,0.09)	-0.06 (-0.21,0.09)	-0.07 (-0.22,0.08)
DEHP metabolites	-0.07 (-0.23,0.09)	-0.06 (-0.23,0.10)	-0.08 (-0.24,0.09)
DNOP metabolites	0.01 (-0.11,0.14)	0.00 (-0.12,0.13)	-0.01 (-0.13,0.12)
Total bisphenols	-0.08 (-0.25,0.08)	-0.09 (-0.25,0.08)	-0.08 (-0.25,0.08)
BPA	-0.10 (-0.26,0.07)	-0.10 (-0.27,0.07)	-0.09 (-0.26,0.08)
BPS	0.11 (-0.05,0.27)	0.12 (-0.04,0.28)	0.11 (-0.06,0.27)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood glucose at 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Basic model includes child´s sex and age. Confounder model includes maternal educational level and child´s sex, age, diet quality score and television watching time. Prenatal exposure model additionally includes maternal exposure to phthalate metabolites and bisphenols during pregnancy (natural log-transformed pregnancy-averaged urinary concentrations in µmol/g creatinine).

Table S7. Associations of phthalate metabolites and bisphenols urinary concentrations with blood pressure in childhood, prenatal exposure model[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores					
	Systolic blood pressure			Diastolic blood pressure		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.03 (-0.14,0.08)	0.07 (-0.05,0.18)	0.14 (0.03,0.26)*	0.01 (-0.10,0.11)	0.02 (-0.10,0.14)	0.08 (-0.06,0.23)
LMW phthalate	-0.01 (-0.10,0.08)	0.03 (-0.07,0.13)	0.05 (-0.05,0.15)	0.03 (-0.06,0.12)	0.00 (-0.10,0.11)	0.01 (-0.12,0.13)
HMW phthalate	0.10 (-0.02,0.21)	0.14 (0.02,0.26)*	0.07 (-0.06,0.19)	0.13 (0.01,0.24)*	0.10 (-0.03,0.23)	0.04 (-0.11,0.19)
DEHP metabolites	0.07 (-0.06,0.20)	0.11 (-0.02,0.25)	0.08 (-0.06,0.21)	0.10 (-0.02,0.23)	0.05 (-0.09,0.19)	0.03 (-0.13,0.20)
DNOP metabolites	0.06 (-0.03,0.15)	0.10 (0.01,0.20)*	0.07 (-0.03,0.16)	0.08 (-0.01,0.17)	0.09 (-0.01,0.19)	0.04 (-0.08,0.16)
Total bisphenols	0.01 (-0.12,0.14)	-0.00 (-0.14,0.13)	-0.03 (-0.17,0.11)	-0.12 (-0.25,0.01)	0.02 (-0.12,0.16)	0.19 (0.02,0.35)*
BPA	-0.01 (-0.14,0.13)	-0.00 (-0.14,0.14)	-0.02 (-0.16,0.12)	-0.12 (-0.25,0.01)	0.02 (-0.13,0.16)	0.18 (0.01,0.35)*
BPS	0.09 (-0.04,0.22)	0.07 (-0.07,0.21)	-0.01 (-0.14,0.13)	-0.05 (-0.18,0.08)	0.08 (-0.07,0.22)	0.14 (-0.03,0.31)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood blood pressure at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and exposure to phthalate metabolites and bisphenols during pregnancy (natural log-transformed pregnancy-averaged urinary concentrations in µmol/g creatinine) and child sex, age, height, diet quality score and television watching time. *p-value < 0.05.

Table S8. Associations of phthalate metabolites and bisphenols urinary concentrations with insulin and lipids profile in childhood, prenatal exposure model[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores											
	Insulin			Total Cholesterol			HDL Cholesterol			Triglycerides		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.06 (-0.18,0.06)	-0.02 (-0.16,0.11)	0.05 (-0.16,0.25)	0.05 (-0.08,0.17)	0.09 (-0.04,0.22)	-0.03 (-0.14,0.08)	0.07 (-0.05,0.18)	-0.04 (-0.17,0.09)	-0.07 (-0.20,0.05)	0.08 (-0.04,0.20)	0.07 (-0.06,0.20)	-0.02 (-0.21,0.17)
LMW phthalate	-0.04 (-0.15,0.06)	0.07 (-0.05,0.19)	0.09 (-0.09,0.27)	0.06 (-0.04,0.17)	0.09 (-0.03,0.21)	-0.03 (-0.13,0.06)	0.01 (-0.09,0.11)	-0.11 (-0.23,0.00)	-0.09 (-0.20,0.02)	0.09 (-0.01,0.20)	0.10 (-0.02,0.22)	-0.02 (-0.19,0.16)
HMW phthalate	-0.07 (-0.20,0.06)	-0.02 (-0.16,0.13)	0.08 (-0.15,0.30)	0.02 (-0.11,0.16)	0.02 (-0.13,0.16)	-0.01 (-0.13,0.11)	0.07 (-0.06,0.20)	0.02 (-0.13,0.16)	-0.09 (-0.22,0.04)	-0.02 (-0.15,0.12)	0.13 (-0.02,0.28)	0.15 (-0.06,0.36)
DEHP metabolites	-0.08 (-0.23,0.07)	0.02 (-0.14,0.18)	0.11 (-0.14,0.36)	0.03 (-0.12,0.18)	0.05 (-0.11,0.20)	0.00 (-0.13,0.13)	0.09 (-0.05,0.23)	0.06 (-0.10,0.22)	-0.02 (-0.17,0.13)	0.03 (-0.12,0.18)	0.18 (0.02,0.34)*	0.13 (-0.10,0.36)
DNOP metabolites	-0.03 (-0.14,0.07)	-0.02 (-0.14,0.11)	0.05 (-0.14,0.24)	0.02 (-0.09,0.12)	-0.04 (-0.16,0.09)	-0.00 (-0.10,0.10)	0.01 (-0.09,0.11)	-0.09 (-0.21,0.03)	-0.17 (-0.28,-0.06)*†	-0.09 (-0.19,0.02)	0.06 (-0.07,0.18)	0.19 (0.01,0.37)*
Total bisphenols	-0.03 (-0.18,0.13)	-0.17 (-0.33,-0.01)*	-0.15 (-0.40,0.11)	0.10 (-0.07,0.26)	-0.01 (-0.17,0.15)	-0.12 (-0.26,0.02)	0.14 (-0.01,0.29)	-0.00 (-0.16,0.15)	-0.08 (-0.23,0.08)	0.04 (-0.12,0.20)	-0.05 (-0.20,0.11)	-0.13 (-0.38,0.11)
BPA	-0.02 (-0.19,0.14)	-0.19 (-0.35,-0.03)*	-0.17 (-0.43,0.09)	0.06 (-0.11,0.22)	-0.05 (-0.21,0.11)	-0.12 (-0.26,0.02)	0.16 (0.00,0.32)*	0.00 (-0.16,0.16)	-0.08 (-0.24,0.07)	0.05 (-0.12,0.21)	-0.01 (-0.17,0.16)	-0.09 (-0.34,0.16)
BPS	-0.01 (-0.17,0.14)	0.06 (-0.10,0.22)	0.09 (-0.15,0.33)	0.13 (-0.02,0.28)	0.04 (-0.12,0.20)	-0.04 (-0.17,0.09)	-0.03 (-0.18,0.11)	-0.03 (-0.18,0.13)	0.04 (-0.10,0.18)	0.11 (-0.05,0.26)	-0.09 (-0.25,0.07)	-0.15 (-0.38,0.08)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood insulin and lipids profile at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and exposure to phthalate metabolites and bisphenols during pregnancy (natural log-transformed pregnancy-averaged urinary concentrations in µmol/g creatinine) and child sex, age, diet quality score and television watching time. *p-value < 0.05.
† Result remained significant after multiple testing correction.

Table S9. Associations of phthalate metabolites and bisphenols urinary concentrations with blood pressure in childhood, sensitivity analyses[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores			
	Systolic blood pressure		Diastolic blood pressure	
	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years
Phthalic acid	0.11 (0.02,0.21)*	0.11 (0.01,0.20)*	0.03 (-0.08,0.15)	0.04 (-0.07,0.16)
LMW phthalate	0.04 (-0.05,0.12)	0.04 (-0.04,0.12)	0.00 (-0.09,0.10)	0.01 (-0.09,0.10)
HMW phthalate	0.11 (0.00,0.21)*	0.09 (-0.02,0.19)	0.06 (-0.07,0.18)	0.07 (-0.06,0.19)
DEHP metabolites	0.10 (-0.01,0.21)	0.08 (-0.04,0.19)	0.02 (-0.12,0.15)	0.03 (-0.10,0.17)
DNOP metabolites	0.08 (0.00,0.16)*	0.08 (-0.01,0.16)	0.08 (-0.02,0.17)	0.08 (-0.02,0.17)
Total bisphenols	-0.03 (-0.15,0.08)	-0.04 (-0.15,0.08)	0.06 (-0.08,0.19)	0.06 (-0.07,0.20)
BPA	-0.03 (-0.15,0.09)	-0.03 (-0.15,0.09)	0.05 (-0.09,0.19)	0.06 (-0.09,0.20)
BPS	0.03 (-0.08,0.15)	0.01 (-0.10,0.13)	0.10 (-0.04,0.23)	0.10 (-0.04,0.23)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood blood pressure at 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, height, diet quality score, television watching time and additionally for the corresponding outcome at 6 years. *p-value < 0.05.

Table S10. Associations of phthalate metabolites and bisphenols urinary concentrations with insulin and lipids profile in childhood, sensitivity analyses[1]

Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores							
	Insulin		Total Cholesterol		HDL Cholesterol		Triglycerides	
	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.03 (-0.18,0.13)	-0.03 (-0.18,0.13)	-0.01 (-0.11,0.09)	-0.01 (-0.10,0.09)	-0.06 (-0.17,0.05)	-0.06 (-0.17,0.05)	0.03 (-0.11,0.17)	0.03 (-0.12,0.17)
LMW phthalate	0.05 (-0.09,0.18)	0.04 (-0.09,0.17)	-0.00 (-0.09,0.08)	0.00 (-0.08,0.09)	-0.11 (-0.20,-0.02)*	-0.11 (-0.20,-0.02)*	0.10 (-0.03,0.22)	0.09 (-0.04,0.22)
HMW phthalate	0.01 (-0.15,0.18)	0.00 (-0.16,0.17)	0.01 (-0.10,0.12)	0.01 (-0.09,0.12)	-0.04 (-0.16,0.08)	-0.04 (-0.16,0.08)	0.16 (0.01,0.32)*	0.15 (-0.00,0.31)
DEHP metabolites	0.04 (-0.14,0.23)	0.03 (-0.15,0.22)	0.03 (-0.08,0.15)	0.04 (-0.08,0.15)	0.03 (-0.11,0.16)	0.02 (-0.11,0.16)	0.20 (0.03,0.37)*	0.19 (0.02,0.36)*
DNOP metabolites	0.01 (-0.13,0.16)	0.01 (-0.14,0.15)	-0.00 (-0.09,0.09)	-0.00 (-0.09,0.09)	-0.14 (-0.24,-0.04)*†	-0.14 (-0.24,-0.05)*†	0.10 (-0.03,0.24)	0.10 (-0.04,0.23)
Total bisphenols	-0.15 (-0.34,0.04)	-0.15 (-0.34,0.04)	-0.08 (-0.20,0.04)	-0.08 (-0.20,0.04)	0.01 (-0.13,0.15)	0.01 (-0.13,0.15)	-0.14 (-0.32,0.04)	-0.14 (-0.32,0.04)
BPA	-0.19 (-0.38,0.01)	-0.19 (-0.38,0.01)	-0.09 (-0.21,0.04)	-0.09 (-0.21,0.04)	0.00 (-0.14,0.15)	0.01 (-0.14,0.15)	-0.09 (-0.28,0.09)	-0.09 (-0.28,0.09)
BPS	0.09 (-0.09,0.27)	0.08 (-0.11,0.26)	-0.03 (-0.15,0.08)	-0.03 (-0.15,0.08)	0.03 (-0.09,0.16)	0.03 (-0.10,0.16)	-0.11 (-0.28,0.06)	-0.12 (-0.29,0.05)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood insulin and lipids profile at 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Models are adjusted for maternal educational level and child sex, age, diet quality score, television watching time and additionally for the corresponding outcome at 6 years. *p-value < 0.05. † Result remained significant after multiple testing correction.

Table S11. Associations of phthalate metabolites and bisphenols urinary concentrations with blood pressure in childhood, basic models[1]

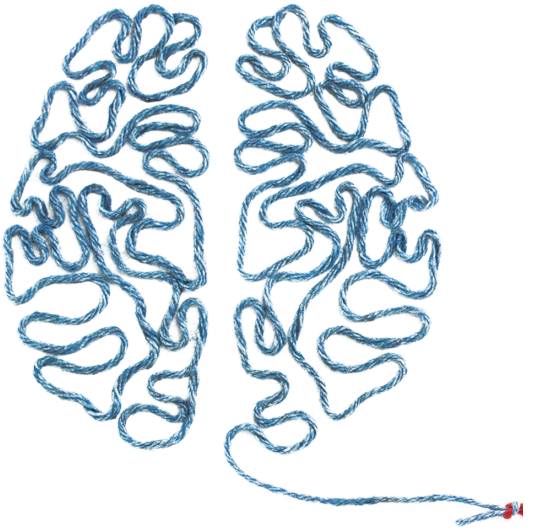
Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores					
	Systolic blood pressure			Diastolic blood pressure		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.02 (-0.13,0.09)	0.07 (-0.04,0.18)	0.14 (0.03,0.25)*	0.03 (-0.08,0.13)	0.03 (-0.09,0.14)	0.08 (-0.06,0.22)
LMW phthalate	0.02 (-0.07,0.11)	0.05 (-0.05,0.14)	0.04 (-0.05,0.14)	0.07 (-0.03,0.16)	0.01 (-0.09,0.11)	-0.01 (-0.13,0.11)
HMW phthalate	0.09 (-0.02,0.21)	0.13 (0.01,0.25)*	0.07 (-0.05,0.19)	0.13 (0.02,0.25)*	0.09 (-0.04,0.21)	0.03 (-0.12,0.18)
DEHP metabolites	0.06 (-0.07,0.18)	0.10 (-0.03,0.23)	0.08 (-0.05,0.21)	0.10 (-0.02,0.23)	0.04 (-0.09,0.18)	0.02 (-0.14,0.18)
DNOP metabolites	0.07 (-0.02,0.16)	0.10 (0.01,0.20)*	0.06 (-0.04,0.15)	0.10 (0.00,0.19)*	0.09 (-0.01,0.19)	0.04 (-0.08,0.16)
Total bisphenols	-0.01 (-0.13,0.12)	-0.03 (-0.16,0.10)	-0.04 (-0.17,0.09)	-0.13 (-0.26,-0.01)*	0.00 (-0.14,0.14)	0.19 (0.03,0.35)*
BPA	-0.02 (-0.16,0.11)	-0.03 (-0.17,0.10)	-0.02 (-0.16,0.11)	-0.13 (-0.27,-0.00)*	-0.00 (-0.14,0.14)	0.18 (0.01,0.35)*
BPS	0.08 (-0.05,0.21)	0.05 (-0.08,0.19)	-0.03 (-0.17,0.10)	-0.06 (-0.19,0.07)	0.07 (-0.07,0.21)	0.16 (-0.01,0.32)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood blood pressure at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Basic models include child´s sex, age and height. *$p < 0.05$.

Table S12. Associations of phthalate metabolites and bisphenols urinary concentrations with insulin and lipids profile in childhood, basic models[1]

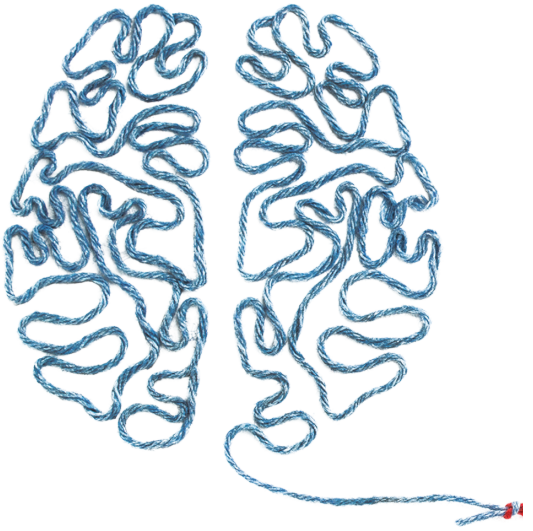
Endocrine disrupting chemicals urinary concentrations	Difference (95% Confidence Interval) in z-scores											
	Insulin			Total Cholesterol			HDL Cholesterol			Triglycerides		
	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years	6 years	10 years	Change from 6 to 10 years
Phthalic acid	-0.05 (-0.17,0.07)	-0.03 (-0.17,0.10)	0.03 (-0.17,0.23)	0.04 (-0.08,0.16)	0.09 (-0.04,0.22)	-0.03 (-0.13,0.08)	0.06 (-0.05,0.18)	-0.04 (-0.17,0.09)	-0.09 (-0.21,0.03)	0.07 (-0.05,0.19)	0.06 (-0.07,0.19)	0.01 (-0.18,0.19)
LMW phthalate	-0.03 (-0.13,0.07)	0.04 (-0.08,0.16)	0.05 (-0.13,0.23)	0.06 (-0.04,0.16)	0.09 (-0.02,0.20)	-0.03 (-0.12,0.06)	-0.00 (-0.10,0.10)	-0.12 (-0.23,-0.00)*	-0.10 (-0.20,0.01)	0.09 (-0.02,0.19)	0.09 (-0.03,0.20)	0.00 (-0.16,0.17)
HMW phthalate	-0.06 (-0.18,0.07)	-0.01 (-0.15,0.14)	0.10 (-0.12,0.32)	0.02 (-0.11,0.15)	0.03 (-0.11,0.18)	0.00 (-0.12,0.12)	0.09 (-0.04,0.21)	0.04 (-0.10,0.18)	-0.10 (-0.23,0.03)	-0.02 (-0.15,0.11)	0.13 (-0.01,0.27)	0.16 (-0.04,0.37)
DEHP metabolites	-0.07 (-0.22,0.07)	0.02 (-0.14,0.18)	0.13 (-0.11,0.37)	0.03 (-0.11,0.17)	0.07 (-0.09,0.22)	0.02 (-0.11,0.14)	0.11 (-0.03,0.24)	0.09 (-0.06,0.24)	-0.04 (-0.18,0.10)	0.02 (-0.12,0.17)	0.17 (0.02,0.33)*	0.14 (-0.08,0.37)
DNOP metabolites	-0.03 (-0.13,0.08)	0.00 (-0.12,0.13)	0.06 (-0.13,0.25)	0.01 (-0.09,0.12)	-0.02 (-0.14,0.10)	0.00 (-0.10,0.10)	0.02 (-0.08,0.12)	-0.08 (-0.20,0.04)	-0.17 (-0.28,-0.07)*†	-0.09 (-0.19,0.02)	0.07 (-0.06,0.19)	0.20 (0.03,0.37)*
Total bisphenols	-0.01 (-0.17,0.14)	-0.15 (-0.31,0.00)	-0.17 (-0.42,0.09)	0.10 (-0.05,0.25)	-0.01 (-0.16,0.15)	-0.12 (-0.25,0.01)	0.17 (0.03,0.32)*	-0.01 (-0.16,0.15)	-0.07 (-0.22,0.08)	0.03 (-0.13,0.18)	-0.05 (-0.21,0.10)	-0.14 (-0.37,0.10)
BPA	-0.02 (-0.18,0.14)	-0.18 (-0.35,-0.02)*	-0.19 (-0.44,0.07)	0.07 (-0.09,0.23)	-0.04 (-0.20,0.11)	-0.12 (-0.26,0.01)	0.20 (0.05,0.35)*	-0.00 (-0.16,0.16)	-0.08 (-0.23,0.08)	0.03 (-0.13,0.19)	-0.02 (-0.18,0.15)	-0.09 (-0.33,0.15)
BPS	-0.01 (-0.16,0.14)	0.06 (-0.10,0.22)	0.05 (-0.19,0.29)	0.14 (-0.01,0.28)	0.03 (-0.12,0.18)	-0.05 (-0.17,0.08)	-0.01 (-0.16,0.13)	-0.04 (-0.19,0.11)	0.04 (-0.11,0.17)	0.09 (-0.06,0.24)	-0.08 (-0.24,0.07)	-0.14 (-0.36,0.08)

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in z-scores childhood insulin and lipids profile at 6 and 10 years and the change in the outcomes in z-scores from 6 to 10 years for an interquartile range increase in each natural log-transformed phthalate metabolites and bisphenols urinary concentrations in µmol/g creatinine. Change from 6 to 10 years correspond to the difference in the outcome between 6 and 10 years. Basic models include child´s sex and age. *p < 0.05. †Result remained significant after multiple testing correction.



Chapter 3

Childhood brain health



Chapter 3.1

Maternal cardiovascular health in early pregnancy and childhood brain structure

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Adapted from: J Am Heart Assoc. 2022;11(19):e026133.

ABSTRACT

Background: Poor cardiovascular health during pregnancy has been associated with adverse neurocognitive outcomes in the offspring. We examined the associations of maternal cardiovascular health factors with brain structure, in 10-year-old children.

Methods and Results: We included 2,797 mother-offspring pairs from The Generation R Study. Maternal body mass index, gestational weight gain, blood pressure, insulin, glucose and lipids blood concentrations were obtained in early pregnancy. Childhood structural brain measures, including global metrics of brain tissue volumes and white matter microstructure, were quantified by magnetic resonance imaging at 10 years. As compared to offspring of mothers with normal weight, those of mothers with underweight had smaller total brain volume (difference −28.99 (95% Confidence Interval (CI) −56.55,−1.45)cm^3). Similarly, as compared to offspring of mothers with gestational weight gain between 25^{th}-75^{th} percentile, those of mothers with gestational weight loss or no gestational weight gain (<25^{th} percentile), had smaller total brain volume (difference −13.07(95% CI −23.82,−2.32)cm^3). Also, higher maternal diastolic blood pressure in early pregnancy was associated with lower offspring white matter mean diffusivity (MD) (difference −0.07(95% CI −0.11,−0.02)standard deviation score(SDS)). After multiple testing correction, only the association of maternal diastolic blood pressure with lower offspring white matter MD remained statistically significant. No associations were observed of maternal insulin, glucose and lipids concentrations with childhood brain outcomes.

Conclusions: Our findings suggest that maternal cardiovascular health during pregnancy might be related to offspring brain development on the long term. Future studies are needed to replicate our findings, and to explore the causal nature of the associations.

INTRODUCTION

Cardiovascular disease is a major public health problem and is the leading cause of mortality and morbidity in the general adult population worldwide.[1] In particular, an adverse cardiovascular health status in pregnant women may lead to pregnancy complications and have long-term consequences for the offspring.[2-4] A suboptimal intrauterine environment, due to adverse maternal cardiovascular risk factors, can lead to placental dysfunction, inflammation and changes in various metabolic processes, potentially altering fetal brain development, which may subsequently influence brain health in later life.[5-7] The existing literature suggests that adverse maternal body fat measures and cardiovascular risk factors during pregnancy can potentially affect brain development, behavior and cognition in children. For example, maternal obesity, hypertension and diabetes during pregnancy have been associated with adverse neurodevelopmental and cognitive outcomes in offspring including lower intelligence, executive functioning, developmental delay, and mental and behavioral disorders.[8-17] Also, a growing body of evidence suggests that not only offspring exposed to maternal obesity, but offspring of mothers with underweight may have impaired intellectual development.[18-20] In contrary, a prospective study among 5,191 mother-offspring pairs from the ALSPAC cohort suggested a positive association of gestational weight gain with better cognitive outcomes in offspring.[21] Though some previous studies have focused on neurocognitive outcomes, the associations of maternal cardiovascular risk factors with offspring brain structure were rarely explored. A previous neuroimaging study showed that offspring of pregnancies complicated by preeclampsia, as compared to offspring of uncomplicated pregnancies, exhibited enlarged brain regional volumes of the cerebellum, temporal lobe and amygdala at 7-10 years old.[22] In addition to brain structural changes, a functional magnetic resonance imaging (MRI) study among 91 children aged 7–11 years suggested an association between children exposed to gestational diabetes mellitus and increased hypothalamic blood flow in response to glucose.[23] Studies performed thus far have mainly focused on maternal clinically manifest diseases and do not allow for clear conclusions about the associations of maternal body fat measures and cardiovascular risk factors with offspring brain development. Recent results from our own research group, the Generation R Study, showed that higher maternal pre-pregnancy body mass index (BMI) was associated with differences in white matter microstructure in offspring aged 10 years.[24] In the current study, we aimed to extend previous findings including several cardiovascular risk factors as well as focusing in early pregnancy as a sensitive period since it largely reflects maternal fat deposition and cardiovascular profile and to a lesser extent growth changes of the fetus, placenta and uterus which mostly occurs in mid and late pregnancy.[25]

Therefore, in this large population-based cohort study of 2,797 mother-offspring pairs, we investigated the associations of maternal cardiovascular health factors, including BMI, gestational weight gain, blood pressure, and insulin, glucose, and lipids concentrations in

early pregnancy with childhood brain structure at 10 years of age. We used a hierarchical approach that involved global measures of brain volume and white matter microstructure without defining specific regions of interest as there was no a priori hypothesis.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort from early pregnancy onwards. Approval was obtained from the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, the Netherlands (MEC 198.782/2001/31), and the

procedures followed were in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants.[26] Pregnant women were enrolled between April 2002 and January 2006. In total, 5,706 mothers and their singleton children attended the study visit at 10 years, of whom information about maternal cardiovascular health factors in early pregnancy was available in 5,169 subjects. Next, we excluded 1,696 children who did not participate in the neuroimaging assessment and an additional 676 children without sufficient quality of neuroimaging data. The final population for analysis comprised 2,797 mother-offspring pairs with structural MRI and 2,645 with useable Diffusion Tensor Imaging (DTI) outcomes (for Flowchart see **Figure S1** in Supplementary Materials).

Early pregnancy cardiovascular health factors

Maternal cardiovascular health factors were all measured in early pregnancy (gestational age 13.1 weeks [95% range 9.8, 17.4]) and included information on maternal BMI, gestational weight gain, blood pressure, and non-fasting insulin, glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides concentrations.[27]

Maternal BMI (kg/m^2) in early pregnancy was calculated from height and weight, both measured without shoes and heavy clothing. Maternal BMI was categorized into clinical categories according to World Health Organization cutoffs (underweight [<18.5 kg/m^2], normal weight [18.5–24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obesity [≥30.0 kg/m^2]).[28] Gestational weight gain in early pregnancy was calculated as the difference between weight at enrolment and pre-pregnancy weight obtained by questionnaire.[29] We divided weight gain by gestational age to obtain the weight gain per week.[30] Further, we categorized gestational weight gain per week into below 25th percentile (ranging from weight loss to 0 weight gain), between 25th – 75th percentile (ranging from 0.007 to 0.286 kg/week), and above 75th percentile (weight gain greater than 0.286 kg/week). Blood pressure was measured in the right upper arm, in sitting position, with the validated Omron 907® automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands) by well-trained staff. Systolic and diastolic blood pressure were averaged from 2 measurements with

one-minute interval.[31] Insulin, glucose, total cholesterol, HDL-cholesterol and triglycerides concentrations were measured from non-fasting blood samples. Sample processing and storage procedures have been previously described.[32]

Brain imaging at 10 years

Brain scans were obtained on a single 3-Tesla scanner (General Electric MR750w, Milwaukee, WI, USA) using the same sequence protocol for all children. Following a three-plane localizer scan, a high resolution 3D T1-weighted scan was acquired with an inversion recovery fast-spoiled gradient recalled sequence (parameters: TR=8.77 ms, TE=3.4ms, TI=600ms, flip angle=10°, FOV=220 mm x 220 mm, acquisition matrix=220 x 220, slice thickness=1 mm, number of slices=230).[33] Images were processed using FreeSurfer, version 6.0 (http://surfer.nmr.mgh.harvard.edu/).[34] The quality of FreeSurfer output was visually inspected, and images with artefacts and insufficient quality were excluded. Global metrics of volume, including total brain volume, total gray matter volume and total white matter volume were extracted.

DTI outcomes were obtained using an echo-planar sequence with three b=0 scans and 35 diffusion-weighted images (b=1000 s/mm2) with the parameters: TR=12,500 ms, TE=72.8 ms, FOV=240 mm x 240 mm, acquisition matrix=120 x 120, slice thickness=2 mm, number of slices=65. DTI data were processed with the FMRIB Software Library (FSL) and the Camino diffusion MRI toolkit.[35,36] The diffusion tensor was fit at each voxel, and common scalar metrics including global fractional anisotropy (FA),mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were computed. FA describes the degree of anisotropic diffusion and is highly sensitive to microstructural changes, and MD describes the average diffusion in all directions.[37,38] White matter tracts, were determined using fully-automated probabilistic fiber tractography.[37,39] Average FA and MD were calculated for each tract. Diffusion image quality was assessed by manual and automated inspection.

Covariates

Information on maternal age, ethnicity, educational level, parity, smoking habits, alcohol consumption, and folic acid supplement use was obtained by questionnaires during pregnancy. Maternal psychopathology, particularly pregnancy-specific anxiety, was assessed by an adapted version of the Pregnancy Outcome Questionnaire at around 12 weeks of gestation.[40,41] Child sex and date of birth, from which age at neuroimaging assessment was calculated, were available from medical records.

Statistical analysis

A non-response analysis was performed to test for potential differences in baseline characteristics between participants and non-participants using Pearson's chi-square tests, independent sample t-tests and Mann-Whitney tests. We assessed the associations of maternal cardiovascular health factors in early pregnancy with childhood global volumetric measures

including total brain, total gray matter and total white matter volume, and overall white matter microstructure measures using linear regression analyses. Non-linearity of the relationship was assessed using generalized additive models. Since the associations of maternal BMI and gestational weight gain with brain outcomes in 10-year-old children were not linear, we present the results for the BMI categories (underweight, normal weight, overweight and obesity) and gestational weight gain categories (< 25^{th} percentile, between 25^{th} -75^{th}, and > 75^{th} percentile). For all analyses, we present a basic model including child´s sex and age at outcome measurements, and a confounder-adjusted model, which additionally included maternal age, ethnicity, educational level, parity, pregnancy-specific anxiety, smoking habits, alcohol consumption, and folic acid supplement use. Gestational weight gain and blood pressure models were also adjusted for maternal pre-pregnancy BMI and maternal height, respectively. Potential confounders were represented in a directed acyclic graph (**Figure S2** in Supplementary Materials) and were selected based on previous literature and by observing a >10% change in effect estimate.[12, 24] We did not adjust for any birth outcomes (e.g., gestational age or birth weight), as these factors might be an intermediate in the causal pathway of the investigated associations, which could lead to biased results.[42]

To enable comparison of effect sizes of different cardiovascular exposure measures, we constructed standard deviation scores (SDS) ((observed value – mean)/SD). In addition, global white matter microstructure measures were also standardized and if an association with a DTI metric was observed, we performed a follow-up analysis with AD and RD in order to better describe the underlying microstructural properties. Models with global volumetric measures as outcomes were not standardized, due to clinically interpretability in cm^3, nor adjusted for intracranial volume, given the high correlation (r >0.89). Based on previous literature, we tested for statistical interactions with child sex in all models, but none of the interaction terms was significant.[18] As sensitivity analyses, we assessed the associations of maternal pre-pregnancy BMI with offspring brain structure at 10 years.

Missing data in covariates (proportion of missing data ranged from 0 to 13.4%, with the exception of folic acid use [22.3%]) were imputed using Markov chain Monte Carlo approach with use of the fully conditional specification method assuming a nonmonotone missing pattern.[43] We created ten imputed datasets and reported the pooled effect estimates and their 95% Confidence Interval (CI). To minimize false positive findings due to multiple testing we compared each p-value with a threshold defined as 0.05 divided by the effective number of independent tests estimated based on the correlation between the exposures (p-value threshold of 0.0076).[44] We performed all statistical analyses using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS IBM, Chicago, IL, USA).

RESULTS

Subject Characteristics

Both participant characteristics and non-response analyses are presented in **Table 1**. Of all pregnant women, 65% had a European ethnic background, 59.3% were nulliparous and 77.0% never smoked. The non-response analyses showed that as compared to mothers of children without brain MRI data available, mothers of children with brain MRI measurements were slightly older, highly educated, reported less anxiety, more often had a normal weight and gained less weight in early pregnancy.

Table 1. Characteristics of study population and non-response analyses[1]

	Participants (responders) (N= 2,797)	Non-Participants (non-responders) (N= 2,372)	P-value[2]
Maternal characteristics			
Age, mean (SD), years	30.8 (4.8)	30.6 (5.0)	< 0.05
Ethnicity, N(%)			0.16
European	1,786 (65.0)	1,460 (63.1)	
Non-European	962 (35.0)	854 (36.9)	
Education, N (%)			< 0.01
Primary school	181 (6.8)	213 (9.5)	
Secondary school	1,084 (40.8)	988 (44.3)	
Higher education	1,390 (52.4)	1,030 (46.2)	
Parity, N (%)			< 0.05
Nulliparous	1,648 (59.3)	1,322 (56.1)	
Folic acid use, N(%)			< 0.01
Yes	1,737 (79.9)	1,333 (74.6)	
Alcohol consumption, N (%)			< 0.05
Yes	1,238 (50.6)	992 (47.6)	
Smoking, N (%)			< 0.05
Never	1,926 (77.0)	1,574 (74.4)	
Until pregnancy was known	224 (9.0)	184 (8.7)	
Continued during pregnancy	350 (14.0)	358 (16.9)	
Pregnancy-specific anxiety, mean (SD), score	0.8 (0.4)	0.9 (0.4)	< 0.05
Height, mean (SD), cm	168.1 (7.3)	167.4 (7.5)	< 0.01
Pre-pregnancy weight, mean (SD), kg	66.4 (12.5)	66.4 (12.1)	0.96
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.0-34.8)	22.7 (18.2-34.3)	< 0.05
Gestational age in early pregnancy, median (95% range), weeks	13.1 (9.8-17.4)	13.4 (9.9-17.5)	< 0.01
Weight in early pregnancy, mean (SD), kg	69.4 (13.0)	69.5 (12.4)	0.73
BMI, median (95% range), kg/m^2	23.6 (18.8-35.7)	23.9 (18.9-35.6)	< 0.01
BMI categories, n (%)			< 0.05
Underweight	55 (2.0)	38 (1.6)	

Table 1. Characteristics of study population and non-response analyses[1] (*continued*)

	Participants (responders) (N= 2,797)	Non-Participants (non-responders) (N= 2,372)	P-value[2]
Normal weight	1,736 (62.4)	1,394 (59.0)	
Overweight	696 (25.0)	651 (27.6)	
Obesity	294 (10.6)	278 (11.8)	
Weight gain in early pregnancy, mean (SD), kg/week	0.16 (0.2)	0.18 (0.2)	< 0.05
Weight gain in early pregnancy categories, n (%)			< 0.01
< 25th percentile	521 (28.3)	399 (25.4)	
25th – 75th percentile	861 (46.7)	704 (44.8)	
> 75th percentile	460 (25.0)	468 (29.8)	
Systolic blood pressure, mean (SD), mmHg	115.7 (11.9)	115.2 (12.0)	0.18
Diastolic blood pressure, mean (SD), mmHg	67.9 (9.4)	68.0 (9.3)	0.57
Insulin, median (95% range), pmol/L	113.1 (19.8-627.1)	114.7 (19.7-623.0)	0.83
Glucose, mean (SD), mmol/L	4.4 (0.8)	4.4 (0.9)	0.15
Total cholesterol, mean (SD),mmol/L	4.8 (0.9)	4.8 (0.9)	0.89
HDL-cholesterol, mean (SD), mmol/L	1.8 (0.3)	1.8 (0.4)	0.15
Triglycerides, median (95% range), mmol/L	1.3 (0.7-2.7)	1.3 (0.6-2.7)	0.26
Child characteristics			
Sex, N (%)			0.92
Girls	1,415 (50.6)	1,196 (50.4)	
Age at MRI, mean (SD), years	10.1 (0.6)	10.2 (0.8)	< 0.01

[1]Values are means (standard deviatio), medians (95% range) or numbers of subjects (valid %).
[2]P-values for differences in subject characteristics between groups were calculated performing independent sample t-tests for normally distributed continuous variables, Mann-Whitney test for not normally distributed continuous variables and chi-square tests for categorical variables.
BMI indicates body mass index; HDL-cholesterol, high-density lipoprotein-cholesterol; MRI, magnetic resonance imaging; SD, standard deviation.

Early pregnancy body fat measures and offspring brain structure

The results of the basic model showed an inverse U-shaped relation between maternal BMI and brain global volumetric measures in 10-year-old children. As compared to offspring of mothers with normal weight, those of mothers with lower or higher BMI had smaller total brain and total gray matter volumes (all p-values <0.05). Also, as compared to offspring of mothers with gestational weight gain between 25th-75th percentile, those of mothers with gestational weight loss or no gestational weight gain had smaller total brain, total gray and total white matter volumes (all p-values <0.05) (**Table S1** in Supplementary Materials). After adjustment for potential confounders, only the associations of maternal underweight with smaller offspring total brain and total gray matter volumes (differences −28.99 (95% Confidence Interval (CI) −56.55,−1.45) cm^3, −18.12 (95% CI −34.12,−2.12) cm^3, respectively) remained significant. Similarly, the associations of maternal gestational weight loss or no gestational weight gain with smaller offspring total brain and total white matter volumes (differences

−13.07 (95% CI −23.82,−2.32) cm^3, −6.94 (95% CI −12.09,−1.80)cm^3, respectively) also remained significant after adjustment for potential confounders. However, after a multiple testing correction, none of these associations remained statistically significant (**Table 2**). Sensitivity analyses showed similar results (**Table S2** in Supplementary Materials).

Table 2. Maternal body fat measures in early pregnancy and childhood brain structure at 10 years[1]

Maternal body fat measures	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
BMI categories (N=2,781)					
Underweight (N=55)	-28.99 (-56.55,-1.45)*	-18.12 (-34.12,-2.12)*	-10.85 (-24.08,2.38)	-0.08 (-0.43,0.27)	0.19 (-0.16,0.54)
Normal weight (N=1,736)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (N=696)	-5.38 (-14.53,3.78)	-3.43 (-8.75,1.89)	-1.94 (-6.34,2.46)	-0.02 (-0.13,0.09)	0.01 (-0.10,-0.12)
Obesity (N=294)	0.31 (-12.75,13.36)	-2.56 (-10.14,5.02)	2.85 (-3.42,9.12)	-0.00 (-0.16,0.01)	-0.05 (-0.21,0.11)
Gestational weight gain categories (N=1,842)					
< 25th percentile (N=521)	-13.07 (-23.82,-2.32)*	-6.09 (-12.36,0.18)	-6.94 (-12.09,-1.80)**	-0.07 (-0.21,0.06)	0.03 (-0.10,0.16)
25th-75th percentile (N=861)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
> 75th percentile (N=460)	-4.50 (-15.79,6.80)	-5.20 (-11.78,1.38)	0.69 (-4.71,6.10)	-0.02 (-0.16,0.12)	0.02 (-0.12,0.16)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment and maternal age, ethnicity, educational level, parity, pregnancy-specific anxiety and smoking, alcohol and folic acid use during pregnancy. Gestational weight gain models are additionally adjusted for maternal pre-pregnancy BMI. *p < 0.05. ** p < 0.01.

Early pregnancy cardiovascular measures and offspring brain structure

In the basic model, higher maternal insulin was associated with smaller total brain and total gray matter volumes in offspring aged 10 years (all p-values <0.05). Also, higher maternal HDL-cholesterol was associated with larger offspring total gray matter volume and higher white matter FA (all p-values <0.05) (**Table S3** in Supplementary Materials). After adjustment for potential confounders, these results attenuated into non-significant. In the confounder models, one standard deviation score (SDS) increase in maternal systolic and diastolic blood pressure were associated with lower offspring white matter MD (differences −0.05 (95% CI −0.09,−0.00) SDS, −0.07 (95% CI −0.11,−0.02) SDS, respectively). However, after multiple testing correction, only the association of maternal diastolic blood pressure with lower offspring white matter MD remained statistically significant. A follow-up analysis showed that higher maternal systolic and diastolic blood pressure were associated with lower offspring white

matter AD and maternal diastolic blood pressure were additionally associated with lower RD (all p-values <0.05) (**Table S4** in Supplementary Materials). No associations were observed between maternal glucose, total cholesterol and triglycerides concentrations and any of the offspring brain outcomes (**Table 3**).

Table 3. Maternal cardiovascular risk factors during pregnancy and childhood brain outcomes at 10 years[1]

Maternal cardiovascular risk factors (SDS)	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total Brain volume	Total Gray Matter	Total White Matter	Fractional Anisotropy	Mean Diffusivity
Systolic blood pressure (N= 2,777)	1.42 (-2.40,5.25)	0.35 (-1.89,2.57)	1.08 (-0.77,2.92)	0.02 (-0.03,0.07)	-0.05 (-0.09,-0.00)*
Diastolic blood pressure (N=2,777)	-2.25 (-6.03,1.52)	-1.76 (-3.95,0.44)	-0.50 (-2.32,1.32)	0.01 (-0.04,0.06)	-0.07 (-0.11,-0.02)** †
Insulin (N=2,113)	-3.18 (-7.56,1.20)	-2.55 (-5.11,0.00)	-0.62 (-2.71,1.47)	-0.01 (-0.06,0.05)	-0.03 (-0.09,0.02)
Glucose (N=2,114)	-2.61 (-6.90,1.68)	-1.78 (-4.29,0.72)	-0.82 (-2.87,1.23)	-0.04 (-0.10,0.01)	0.02 (-0.04,0.07)
Total Cholesterol (N=2,144)	0.64 (-3.69,4.97)	0.19 (-2.34,2.71)	0.44 (-1.64,2.51)	0.04 (-0.02,0.09)	-0.03 (-0.09,0.02)
HDL Cholesterol (N=2,145)	-0.68 (-5.11,3.76)	-0.32 (-2.90,2.26)	-0.38 (-2.49,1.74)	0.03 (-0.03,0.08)	-0.01 (-0.07,0.04)
Triglycerides (N=2,142)	1.31 (-3.09,5.71)	1.11 (-1.45,3.68)	0.18 (-1.92,2.29)	-0.00 (-0.06,0.05)	-0.03 (-0.09,0.02)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment and maternal age, ethnicity, educational level, parity, pregnancy-specific anxiety and smoking, alcohol and folic acid use during pregnancy. Systolic and diastolic blood pressure models are additionally adjusted for maternal height. *p < 0.05. ** p < 0.01. † These results survived multiple comparison correction.

DISCUSSION

In this population-based prospective cohort study, higher maternal diastolic blood pressure in early pregnancy was associated with lower global white matter MD in offspring aged 10 years. Our findings also suggest that as compared to offspring of mothers with normal weight, those of mothers with underweight tended to have smaller total brain and total gray matter volumes. Similarly, maternal gestational weight loss or no gestational weight gain in early pregnancy tended to be associated with smaller offspring total brain and total white matter volumes.

Interpretation of main findings

Poor cardiovascular health profile of mothers during pregnancy have been associated with suboptimal neurodevelopment outcomes in the offspring.[5,7] In early pregnancy, human brain

development begins with the differentiation of the neural progenitor cells and by the end of the embryonic period the rudimentary structures of the brain and central nervous system are already established.[45] During this ongoing complex process, fetal brain growth and development occur rapidly, thus adverse exposures during this period may have long-term consequences for child brain development.

A UK birth cohort among 11,025 5-year-old children and 9,882 7-year-old children showed that maternal pre-pregnancy BMI was negatively associated with children's cognitive performance.[9] Similarly, previous studies have suggested an association between prenatal exposure to maternal obesity and higher risk of intellectual disability and lower executive functioning.[8,10,11] In addition, a recent neuroimaging study including our study group and data from two other European birth cohorts showed that maternal pre-pregnancy BMI was associated with higher FA and lower MD in offspring aged 10 up to 26 years.[24] Similarly, maternal underweight has been associated with impaired intellectual development in offspring.[18,19] Interestingly, results of a longitudinal study showed that both low and high maternal pre-pregnancy BMI were associated with increased risk of delayed mental development among 2-year-old US children.[20] In line with these previous findings, in the current study we found an inverse U-shaped relation between maternal BMI and brain global volumetric measures in offspring aged 10 years. Even when accounting for socioeconomic and lifestyle family factors, the associations of maternal underweight with smaller offspring total brain and total gray matter volumes remained significant. However, after a multiple testing correction, these results attenuated into non-significant. We cannot exclude the possibility that our results might have been underpowered to detect differences by maternal underweight because of the small sample size (n=55).

Literature on the association between gestational weight gain and offspring neurodevelopment is limited and inconsistent. On the one hand, results of a prospective study from the ALSPAC cohort among 5,191 mother-offspring pairs suggested positive associations of gestational weight gain with cognitive development outcomes in offspring aged 4,8 and 16 years.[21] On the other hand, other studies reported no association or inverse association between excessive gestational weight gain and offspring cognitive development.[11,18,46] In the current study, we focused specifically on weight gain in early pregnancy since its largely reflects maternal fat deposition, whereas gestational weight gain in mid and late pregnancy reflects growth of the fetus, placenta and uterus. We observed that as compared to offspring of mothers with gestational weight gain between 25th -75th, those of mothers with maternal gestational weight loss or no gestational weight gain tended to have smaller total brain and total white matter volumes in childhood. However, these results should be interpreted with caution as the effect estimates attenuated after multiple testing correction.

Hypertensive disorders of pregnancy, a group of disorders including chronic hypertension, gestational hypertension and preeclampsia, are key risk factors for pregnancy complications and have been associated to offspring suboptimal mental and neurodevelopmental

outcomes.[12-15] A Finnish prospective study among 4,743 mother-child pairs showed that maternal preeclampsia increase the risk of childhood behavioral and emotional disorders in offspring from birth up to 10.8 years.[15] Previous DTI studies suggests a link between neurodevelopmental disorders and cognitive abilities and differences in white matter microstructure among children and adolescents.[47-49] In the present study, we found an association of higher maternal systolic and diastolic blood pressure with lower offspring global white matter MD and AD. However, only the association of maternal diastolic blood pressure remained statistically significant after multiple testing correction. Although the independent associations of systolic and diastolic blood pressure with cerebrovascular outcomes have been previously demonstrated, we cannot exclude the possibility of these specific results be chance findings.[50,51] Prior work has demonstrated that higher FA and lower MD are linked to white matter maturation and the opposite linked to pathology.[39] Thus, based on these general observations, the association of higher diastolic blood pressure linked to lower MD is counterintuitive. However, several explanations may exist which could explain this finding. First, the diffusion tensor model used in this study is sensitive to complex fiber patters (e.g., crossing fibers). It is possible that in some brain areas, more mature or healthy white matter shows a more complex fiber architecture, which then could manifest as lower FA and higher MD.[52] Another potential example related to accelerated maturation, where under stress the brain develops at a faster pace.[53] It is important to note that these examples are purely speculative, and, as the current study focused on brain morphology and structural connectivity only, caution is warranted regarding potential functional implications of the observed associations.

Diabetes in pregnancy, including gestational diabetes mellitus or pre-gestational diabetes, have been also associated with increased risk of childhood poorer cognitive outcomes.[54-56] A previous functional neuroimaging study among 91 children aged 7–11 years suggested an association between children exposed to gestational diabetes mellitus and increased hypothalamic blood flow in response to glucose which might predict greater adiposity risk. Moreover, previous studies among women with diabetes also suggested an association between maternal lipid and glucose concentrations during pregnancy and poorer performance of the offspring on IQ tests and motor development assessments.[16,17] Contrarily to previous findings, we did not observe associations of maternal insulin, glucose, and lipids concentrations in early pregnancy with childhood brain structure. A possible explanation for our findings is that subtle differences in maternal metabolic risk factors before the onset of a clinically manifest disease may not trigger detectable structural brain changes in offspring.

Several potential mechanisms could explain the reported association of maternal diastolic blood pressure and differences in offspring white matter microstructure. For example, a suboptimal placental perfusion due to high blood pressure in pregnancy can lead to reduced oxygen and nutritional supplies, potentially altering ongoing neurodevelopmental processes that start prenatally and continue throughout childhood, such as altered axonal development or myelination, which may subsequently influence brain health in later life.[57] Other

mechanisms include alterations in immune system and hypothalamic-pituitary-adrenal axis functioning. Previous studies showed that altered hypothalamic-pituitary-adrenal axis and immune system functioning were associated with both hypertensive pregnancy disorders and neurodevelopmental outcomes in offspring.[58-61]

Altogether, the associations of maternal cardiovascular health factors in early pregnancy with brain structure in offspring aged 10 years were largely explained by socioeconomic and lifestyle family factors. Maternal diastolic blood pressure may, independently of these factors, be associated with differences in offspring white matter microstructure. Although the observed associations are small on individual level and should be cautiously interpreted, the results may be important from a developmental perspective since adverse exposures during pregnancy may potentially lead to long-term consequences for offspring brain development. Due to the observational design of this study, we cannot infer causality. Further studies are needed to replicate and validate our findings and to further investigate potential causal pathways and underlying mechanisms of these observed associations.

Strengths and limitations

Study strengths were the prospective, population-based design, the large number of subjects for whom we had detailed information available on maternal measurements and offspring neuroimaging data, and the adjustment for multiple potential confounders. However, this study also has some limitations. First, of all singleton children that attended the study visit at 10 years, 90.6% of their mothers had information on at least one measurement of cardiovascular health in early pregnancy. Only 54.1% of the children had neuroimaging data available at 10 years. Nonresponse could lead to selection bias if the associations of maternal cardiovascular health in early pregnancy with offspring brain outcomes differ between those included and excluded in the analyses. As shown in the non-response analyses, mothers of children with and without brain MRI data available were different regarding the socioeconomic background, lifestyle characteristics, and cardiovascular health profile. We cannot exclude the possibility of selection bias, but we believe it has little influence on our findings since we adjusted for most of these factors.[62] Second, we used BMI cut-offs for non-pregnant adults to create the BMI categories in early pregnancy. This might have resulted in a misclassification of BMI. However, similar results were observed after assessing the associations of maternal pre-pregnancy BMI with childhood brain structure. Also, we relied on a self-reported pre-pregnancy weight to calculate the gestational weight gain in early pregnancy, which might introduce potential misclassification bias, and subsequently could lead to an underestimation of the observed associations. However, it is likely to be limited, due to the good reliability and validity of self-reported pre-pregnancy weight in representing measured weight.[63] Third, weight gain was divided by gestational age to obtain the gestational weight gain per week. While throughout pregnancy weight gain is generally non-linear, assuming a constant weight gain in early pregnancy could have influenced our results. However, this is

probably not likely as little individual variation in weight gain is expected in the first weeks of pregnancy.[64] Fourth, we used non-fasting instead of fasting blood samples, which might lead to less precision in the cardiovascular risk factor assessments. Although non-fasting samples are largely used in population-based studies, they may reduce the accuracy of the exposure measurements. Fifth, since the prevalence of mothers with underweight was low in this study population, the power to detect an association may have been more limited due to lower sample sizes. Finally, although we have adjusted for many potential confounders, unmeasured residual confounding might still be present due to the observational design of the study.

CONCLUSION

Maternal diastolic blood pressure in early pregnancy was associated with lower global white matter diffusivity in offspring aged 10 years. Our findings also suggest that maternal BMI and gestational weight gain in early pregnancy may be associated with offspring brain volumes. Future studies are needed to assess causality and explore whether these associations link to neurocognitive outcomes.

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SUPPLEMENTARY MATERIALS

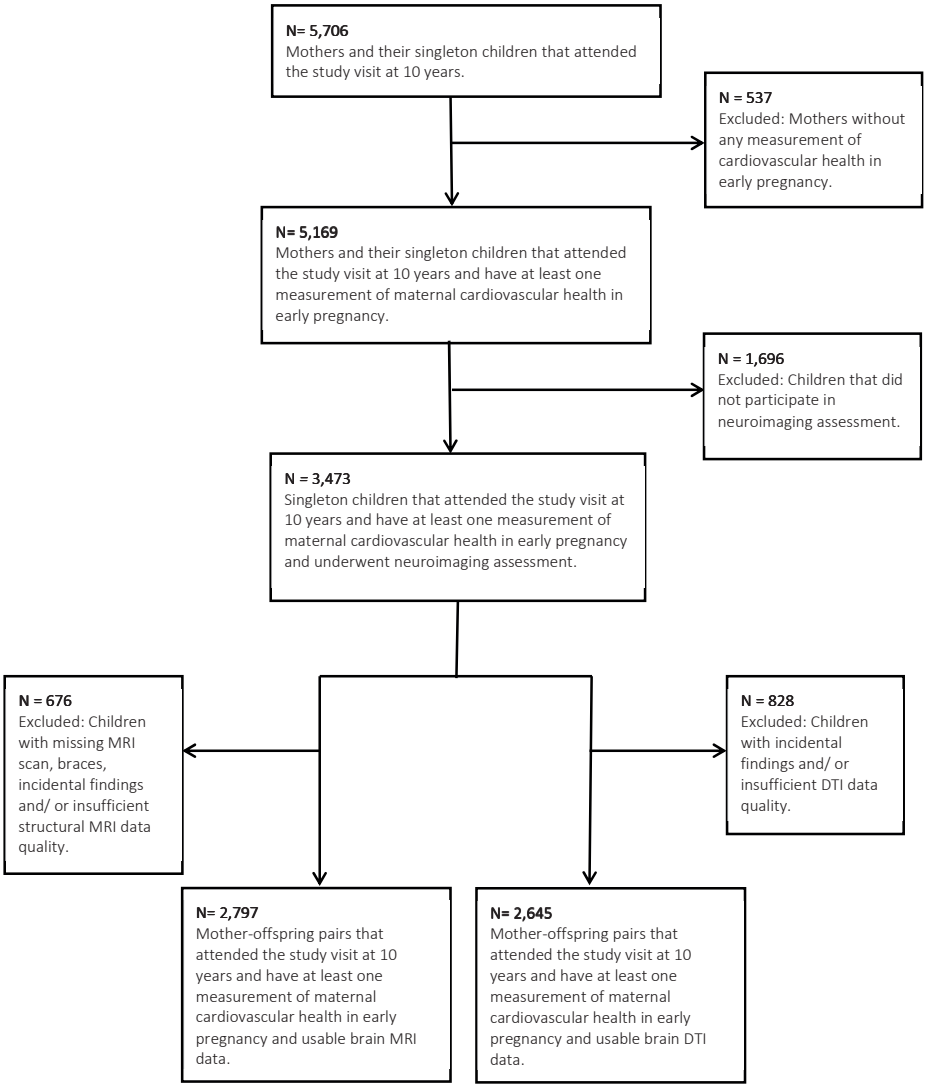


Figure S1. Flowchart of study population

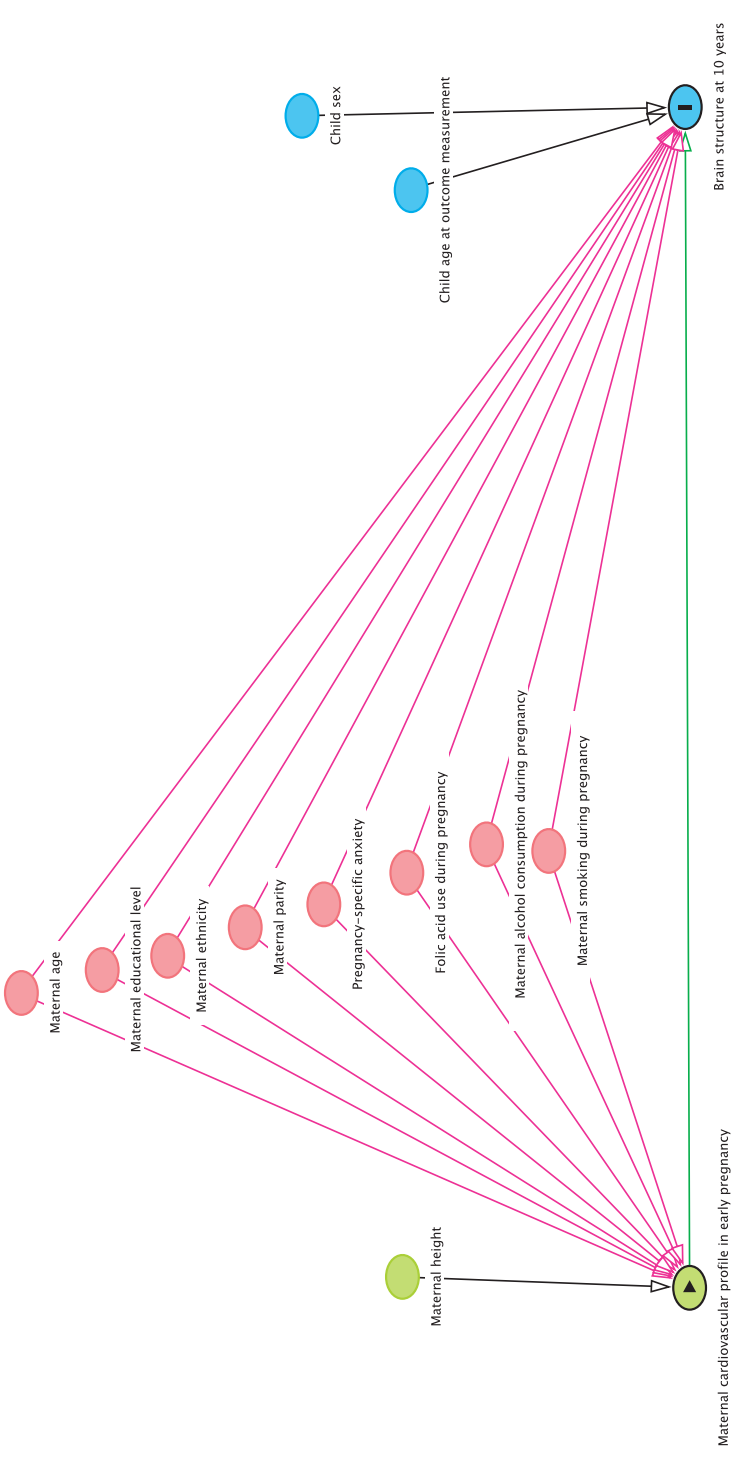


Figure S2. Directed Acyclic Graph showing the hypothesized relationship between maternal cardiovascular risk factors in early pregnancy and childhood brain structure and the covariates

Table S1. Maternal body fat measures in early pregnancy and childhood brain structure at 10 years, basic models[1]

Body mass index	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
BMI categories (N=2,781)					
Underweight (N=55)	-30.30 (-55.65,-4.95)*	-18.59 (-34.50,-3.68)*	-11.72 (-23.57,0.13)	-0.09 (-0.39,0.22)	0.15 (-0.15,0.45)
Normal weight (N=1,736)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (N=696)	-15.95 (-24.25,-7.66)**	-10.63 (-15.51,-5.75)**	-5.32 (-9.19,-1.44)**	-0.06 (-0.16,0.04)	0.01 (-0.08,-0.10)
Obesity (N=294)	-17.58 (-29.24,-5.91)**	-13.87 (-20.73,-7.01)**	-3.73 (-9.18,1.72)	-0.12 (-0.25,0.02)	-0.03 (-0.16,0.11)
Gestational weight gain categories (N=1,842)					
< 25^{th} percentile (N=521)	-17.80 (-27.92,-7.69)**	-10.42 (-16.39,-4.45)**	-7.35 (-12.07,-2.63)**	-0.09 (-0.21,0.03)	0.04 (-0.07,0.16)
25^{th}-75^{th} percentile (N=861)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
> 75^{th} percentile (N=460)	-8.89 (-19.43,1.64)	-7.55 (-13.76,-1.33)*	-1.36 (-6.28,3.56)	-0.05 (-0.18,0.07)	-0.00 (-0.12,0.12)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment. *p < 0.05. ** p < 0.01.

Table S2. Maternal body fat measures in early pregnancy and childhood brain structure at 10 years, sensitivity analyses[1]

Maternal body fat measures	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
BMI categories (N=2,294)					
Underweight (N=96)	-9.27 (-29.98,11.44)	-5.13 (-17.15,6.90)	-4.17 (-14.07,5.73)	-0.10 (-0.36,0.16)	-0.02 (-0.27,0.24)
Normal weight (N=1,615)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (N=422)	-1.97 (-12.64,8.71)	-2.10 (-8.30,4.09)	0.13 (-4.99,5.25)	-0.04 (-0.17,0.09)	-0.02 (-0.15,0.11)
Obesity (N=161)	5.57 (-10.51,21.65)	-0.69 (-10.02,8.65)	6.26 (-1.45,13.98)	0.04 (-0.16,0.23)	-0.08 (-0.28,0.12)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment and maternal age, ethnicity, educational level, parity, pregnancy-specific anxiety and smoking, alcohol and folic acid use during pregnancy. *p < 0.05. ** p < 0.01.

Table S3. Maternal cardiovascular risk factors in early pregnancy and childhood brain structure at 10 years, basic models[1]

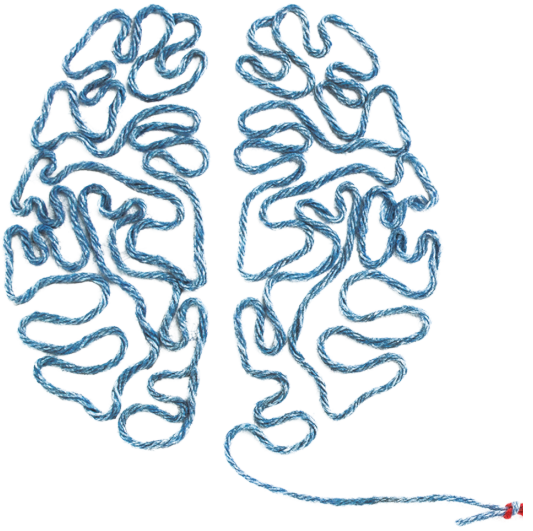
Maternal cardiovascular risk factors (SDS)	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total Brain volume	Total Gray Matter	Total White Matter	Fractional Anisotropy	Mean Diffusivity
Systolic blood pressure (N= 2,777)	1.63 (-1.85,5.11)	0.58 (-1.47,2.62)	1.05 (-0.59,2.69)	0.01 (-0.03,0.06)	-0.3 (-0.07,0.01)
Diastolic blood pressure (N= 2,777)	-1.26 (-4.69,2.16)	-1.23 (-3.24,0.78)	-0.04 (-1.66,1.58)	0.00 (-0.04,0.04)	-0.4 (-0.08,0.00)
Insulin (N=2,113)	-5.71 (-9.75,-1.67)**	-4.25 (-6.62,-1.87)**	-1.46 (-3.34,0.43)	-0.03 (-0.07,0.02)	-0.03 (-0.08,0.02)
Glucose (N=2,114)	-1.91 (-5.97,2.15)	-1.36 (-3.75,1.03)	-0.54 (-2.43,1.35)	-0.04 (-0.09,0.01)	0.01 (-0.04,0.05)
Total Cholesterol (N=2,144)	-1.14 (-5.17,2.89)	-0.70 (-3.07,1.67)	-0.45 (-2.33,1.43)	0.02 (-0.02,0.07)	-0.03 (-0.07,0.02)
HDL Cholesterol (N=2,145)	3.80 (-0.22,7.81)	2.70 (0.34,5.06)*	1.08 (-0.79,2.96)	0.06 (0.01,0.11)*	-0.03 (-0.07,0.02)
Triglycerides (N=2,142)	-3.03 (-7.05,0.99)	-1.55 (-3.91,0.82)	-1.49 (-3.37,0.39)	-0.02 (-0.07,0.02)	-0.02 (-0.06,0.03)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment. Systolic and diastolic blood pressure models are additionally adjusted for maternal height. *p < 0.05. ** p < 0.01.

Table S4. Maternal cardiovascular risk factors in early pregnancy and childhood global white matter microstructure measures at 10 years, follow-up analysis[1]

Maternal cardiovascular risk factors (SDS)	Global white matter microstructure measures (SDS)			
	Fractional Anisotropy	Mean Diffusivity	Radial Diffusivity	Axial Diffusivity
Systolic blood pressure (N= 2,777)	0.02 (-0.03,0.07)	-0.05 (-0.09,-0.00)*	-0.04 (-0.09,0.01)	-0.05 (-0.09,-0.00)*
Diastolic blood pressure (N=2,777)	0.01 (-0.04,0.06)	-0.07 (-0.11,-0.02)**	-0.05 (-0.09,-0.00)*	-0.08 (-0.12,-0.03)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in standard deviation scores of global white matter microstructure measures for maternal cardiovascular risk factors in standard deviation scores. Models are adjusted for child sex and age at the neuroimaging assessment and maternal height, age, ethnicity, educational level, parity, pregnancy-specific anxiety and smoking, alcohol and folic acid use during pregnancy. *p < 0.05. ** p < 0.01.



Chapter 3.2

Fetal and infant growth patterns and brain morphology at age 10 years

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Adapted from: *JAMA Netw Open. 2021 Dec 1;4(12):e2138214.*

ABSTRACT

Importance: Preterm birth and low birth weight are associated with brain developmental and neurocognitive outcomes in childhood; however, not much is known about the specific critical periods in fetal life and infancy for these outcomes.

Objective: To examine the associations of fetal and infant growth patterns with brain morphology at school-age.

Design, setting, and participants: This population-based, prospective cohort study was conducted from February 1 to April 16, 2021, as a part of the Generation R Study in Rotterdam, the Netherlands. The study included 3,098 singleton children born between April 1, 2002, and January 31, 2006.

Exposures: Fetal weight was estimated in the second and third trimester of pregnancy by ultrasound. Infant weight was measured at birth, 6, 12, and 24 months. Fetal and infant weight acceleration or deceleration were defined as a change in standard deviation scores greater than 0.67 between time points. Infant measurements also included peak weight velocity, and age and body mass index reached at adiposity peak.

Main outcome and measures: Brain structure, including global and regional brain volumes, was quantified by magnetic resonance imaging at 10 years.

Results: The study evaluated 3,098 children (mean [SD] follow-up age, 10.1 [0.6] years; 1,557 [50.3%] girls; and 1,753 [57.8%] Dutch). One standard deviation score higher weight gain until the 2nd and 3rd trimester, birth, 6, 12, and 24 months was associated with larger total brain volume, independently of growth during any other age windows (second trimester: 5.7 cm^3; 95%CI, 1.2-10.2 cm^3; third trimester: 15.3 cm^3; 95%CI, 11.0-19.6 cm^3; birth: 20.8 cm^3; 95%CI, 16.4-25.1 cm^3; 6 months: 15.6 cm^3; 95%CI, 11.2-19.9 cm^3; 12 months: 11.3 cm^3; 95%CI, 7.0-15.6 cm^3; and 24 months: 11.1 cm^3; 95%CI, 6.8-15.4 cm^3). Compared to children with normal fetal and infant growth, those with fetal and infant growth deceleration had the smallest total brain volume (–32.5 cm^3; 95%CI, –53.2 to –11.9 cm^3). Children with fetal weight deceleration followed by infant catch-up growth had similar brain volumes as children with normal growth. Higher peak weight velocity and body mass index reached at adiposity peak were associated with larger brain volumes. Similar results were observed for cerebral and cerebellar gray and white matter volumes.

Conclusions and Relevance: This cohort study's findings suggest that both fetal and infant weight growth might be critical for cerebral and cerebellar brain volumes during childhood. Whether these associations link to neurocognitive outcomes should be further studied.

INTRODUCTION

Fetal life and infancy are critical periods for human brain development.[1, 2] During fetal life, and especially in the third trimester of gestation, there is an important growth in brain size. The growth rates of the cortical gray matter and cerebellum peak during this period.[3, 4] Cortical gray matter volume also increases up to 150% in the first year of postnatal life and up to 20% in the second year.[5, 6] Adverse experiences occurring during these periods may permanently influence brain structure and function.[7-9] Currently, evidence to support this hypothesis is mainly based on studies showing that children born preterm or with low birth weight are at risk for suboptimal neurodevelopmental outcomes.[10-19] However, gestational age and weight at birth are merely the end point of fetal development and the starting point for infancy. In infancy, children born small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA) have different growth patterns.[20] Children born SGA tend to have catch-up growth, whereas those born LGA tend to have infant growth deceleration.[20, 21] Observational studies suggest that rapid weight gain in infancy is associated with benefits to later neurocognitive functioning, especially among those born preterm or SGA; however, little is known about its association with brain morphology.[22-24] Population-based studies on the associations of fetal and infant growth patterns with brain structure enable identification of windows of vulnerability for brain development.

We hypothesized that early-life growth patterns might be associated with childhood brain morphology. In a large, population-based, prospective cohort study among 3,098 children, we examined the associations of fetal and infant growth with global and regional brain volumes, measured by magnetic resonance imaging (MRI) at age 10 years.

METHODS

Study design

This cohort study was embedded in the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands. Pregnant women residing in the study area with a delivery date between April 1, 2002 ,and January 31, 2006, were invited to participate. Information on follow-up has been previously described.[25] Written informed consent was obtained for all participants. The Medical Ethics Committee of Erasmus MC approved the study. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. We had information on fetal or infant growth in 9,494 singleton births. Of these, 5,704 children visited the research center at 10 years. All children visiting the center were invited to undergo brain MRI. In total, 3,882 children underwent neuroimaging assessment. Analyses were conducted for 3,098 children for whom we had good-quality neuroimaging (**Figure S1** in Supplementary Materials).

Fetal and infant growth measures

Fetal ultrasonography examinations were performed in each trimester of pregnancy.[25, 26] Gestational age was established by first-trimester ultrasonography.[27] Head circumference, abdominal circumference, and femur length were measured by second- and third- trimester ultrasonography. Fetal weight was estimated using the formula by Hadlock et al.[28] Gestational-age-adjusted standard deviation scores (SDS) were calculated using reference growth curves derived from the same cohort as the current study.[26] At birth, gestational age was categorized into preterm (<37 weeks), term (37-42 weeks) or post-term (>42 weeks).[13] Birth weight was obtained from medical records and was categorized into low (<2500g), normal (2500-4500g) or high (>4500g).[29] We created sex- and gestational age-adjusted SDS for weight at birth within our study population, using the Growth Analyzer 3.5, based on North European reference charts.[30] Children born SGA and LGA were defined as gestational age- and sex-adjusted SDS for birth weight below the 10th and above the 90th percentile, respectively.[31]

Infant weight was measured in community health centers using a mechanical personal scale at approximately 6, 12, and 24 months. Age- and sex-adjusted SDS were obtained using Dutch reference growth charts.[32]

We categorized fetal and infant weight change into 3 groups (growth deceleration, normal growth, and growth acceleration), and created combined variables that reflect 9 different growth patterns. Fetal weight change was defined as growth between the second trimester and birth. Infant weight change was defined as growth from birth to 24 months in 69.4%of the full group. To achieve adequate sample size on subgroup analyses, if weight at 24 months was missing, we calculated the weight change by using 12, or 6 months (20% and 10.6% of the full group, respectively).[31] Growth acceleration or deceleration were defined as a change in SDS greater than 0.67 between time points. This clinically significant change reflects the difference between 2 percentile lines on the growth charts.[33]

Peak weight velocity, reflecting the greatest weight growth in infancy was derived using the Reed1 model by sex on all weight measurements taken from birth to three years of age, including birth weight.[34, 35] To obtain age and BMI reached at adiposity peak, we analysed repeated infant BMI measurements using a cubic mixed-effects model fitted on log(BMI) scale from age 14 days to 1.5 years, adjusted for sex, which showed the best fit to the data.[35, 36] The growth measures are presented in detail in the Supplemental **Table S1**.

Brain outcomes

During the study visit at 10 years, head circumference was measured according standardized procedures.[25] Brain imaging acquisition has been described previously.[37] Briefly, all images were acquired using the same scan protocol on a single 3 Tesla scanner (Discovery MR750,GE Healthcare) using an 8-channel head coil. A high resolution 3D T1-weighted structural scan was obtained with an inversion recovery fast-spoiled gradient recalled sequence. Parameters were as follows: repetition time =8.77ms, echo time=3.4ms, inversion time=600ms, flip angle=10°,

field of view=220mmx220mm, acquisition matrix=220x220, slice thickness=1mm, number of slices=230). At the scanner, T1 image quality was rated using a six-point Likert scale: unusable, poor, fair, good, very good, and excellent. If the initial T1-weighted scan was rated as unusable or poor, the T1 sequence was repeated.[37] Images were processed using FreeSurfer, version 6.0 (http://surfer.nmr.mgh.harvard.edu/). Global and regional volumes, including total brain, and cerebral and cerebellar gray and white matter volumes were extracted. FreeSurfer procedures had good test-retest reliability across scanners.[38] The quality of FreeSurfer output was visually inspected, and all scans rated as unusable (mostly due to motion) were excluded.[39] Scans were reviewed by radiologists for the presence of incidental findings.[40]

Covariates

Information on maternal characteristics, including age, ethnicity, educational level, family income, pre-pregnancy BMI, smoking, alcohol consumption and folic acid supplement use were obtained from questionnaires during pregnancy. Child sex and age at neuroimaging assessment, available from medical records, were included to account for the association of these variables with brain measurements. Intracranial volume was obtained from MRI scans. We calculated BMI at 10 years, using height and weight measured without shoes and heavy clothing. Age- and sex-adjusted SDS for BMI were obtained with Dutch reference growth charts (Growth Analyzer 4.0).[41]

Statistical analysis

First, participants were compared to non-participants to assess possible bias due to loss-to-follow-up. Second, we assessed the associations between the birth outcomes of gestational age and weight and size for gestational age and brain volumes at 10 years using multiple linear regression models. Third, we performed conditional linear regression analysis to identify independent critical periods for early-life growth associated with brain outcomes. Conditional regression analyses take into account correlations between early life growth measures at different ages.[42, 43] We regressed weight at each time point on weight at all previous time points and saved the residual scores, creating noncorrelated growth variables that reflect growth during a certain time period independently of growth during all previous time periods.[44] Fourth, we performed similar regression models to assess the associations of fetal and infant growth patterns, peak weight velocity and BMI and age at adiposity peak with brain volumes at 10 years.

We used a hierarchical approach to examine the associations of fetal and infant growth with brain outcomes. If an association with a subcortical volume was observed, we performed post-hoc analyses of the specific subcortical structures adjusted for intracranial volume. Non-linearity of the association was assessed using generalized additive models and ruled out. For all analyses, we present a basic model including child sex and age at outcome, a confounder model, and a BMI model. Potential confounders were represented in a Directed Acyclic Graph

(**Figure S2** in Supplementary Materials). Confounder models additionally included maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, family income, smoking, alcohol consumption, and folic acid use during pregnancy. The BMI model additionally included childhood sex- and age-adjusted BMI SDS at 10 years. We tested for statistical interactions by child sex in the above analyses, but none of these were significant.[45] Because results were similar when we excluded children born preterm and with low birth weight, we did no restrict or stratify on birth outcomes. Missing data in covariates were multiple-imputed using Markov chain Monte Carlo approach.[46] In total, 30 imputed datasets were created and analyzed, presenting pooled effect estimates with 95% CI (Confidence Interval). No correction for multiple testing was performed, as all exposures and outcomes were highly correlated among each other, and thus a small number of independent tests was expected.[47] Participants were compared with non-participants by using Pearson $\chi 2$ tests, independent-sample t tests, and Mann-Whitney tests. Statistical significance was defined as $p< 0.05$ (2-sided). Statistical analyses were performed using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS IBM, Chicago, IL, USA).

RESULTS

Participant Characteristics

The study evaluated 3,098 children (mean [SD] age, 10.1 [0.6] years; 1,557 [50.3%] girls and 1,541 boys [49.7%]; and 1,753 Dutch [57.8%], 1,026 non-Dutch and non-Western [33.8%], and 253 non-Dutch and Western [8.3%]) (**Table 1**). Non-response analyses showed that compared with mothers of children without brain MRI data available, mothers of children with brain MRI measurements were slightly older, more likely to be of Dutch origin, were highly educated, and smoked less often during pregnancy (**Table S2** in Supplementary Materials).

Birth outcomes

Table 2 shows positive associations of gestational age, weight and size for gestational age at birth with childhood head circumference and brain volumes at 10 years. Compared with children born at term, those born preterm had smaller total brain volume (–28.4 cm^3; 95%CI, –43.9 to –12.8 cm^3). Compared with children with a normal weight at birth, those with low birth weight had smaller total brain volume (–44.9 cm^3; 95%CI, –61.1 to –28.7 cm^3). Compared with children born AGA, those born SGA had smaller total brain volume (–36.6 cm^3; 95%CI, –47.4 to –25.9 cm^3). Similar results were observed for subcortical gray, cerebral and cerebellar gray and white matter volumes. After additional adjustment for childhood BMI, results remained significant (**Table S3** in Supplementary Materials). Post-hoc analyses showed that higher birth weight and size for gestational age were both associated with larger globus pallidus and nucleus accumbens volumes (**Table S4** in Supplementary Materials).

Table 1. Participant Characteristics[1]

Characteristics	Total group (N= 3,098)
Maternal characteristics	
Age at intake, mean (SD), years	31.1 (4.9)
Ethnicity, N(%)	
Dutch	1,753 (57.8)
Non-Dutch, Western	253 (8.3)
Non-Dutch, Non-Western	1,026 (33.8)
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.0-34.8)
Education, N(%)	
Primary school	186 (6.5)
Secondary school	1,156 (40.6)
Higher education	1,506 (52.9)
Monthly household income, US$	
< 1200	330 (13.7)
1200-2000	380 (15.8)
>2000	1,699 (70.5)
Folic acid used, N(%)	
Yes	1,737 (79.9)
Alcohol consumption, N (%)	
Yes	1,238 (50.6)
Smoking, N (%)	
Yes	501 (20.4)
Fetal Characteristics	
Second trimester, median (95% range)	
Gestational age, weeks	20.5 (18.7-23.3)
Estimated fetal weight, g	362 (248-612)
Third trimester, median (95% range)	
Gestational age, weeks	30.4 (28.5-32.7)
Estimated fetal weight, g	1602 (1186-2150)
Birth characteristics	
Gestational age at birth, median (95% range), weeks	40.1 (36.0-42.3)
Sex, N (%)	
Girls	1,557 (50.3)
Birth weight, mean (SD), g	3446 (553)
Infant Characteristics	
At 6 months, median (95% range)	
Age at visit, months	6.2 (5.2-7.9)
Weight, kg	7.8 (6.2-9.7)
At 12 months, median (95% range)	
Age at visit, months	11.0 (10.1-13.0)

Table 1. Participant Characteristics[1] (*continued*)

Characteristics	Total group (N= 3,098)
Weight, kg	9.6 (7.7-11.8)
At 24 months, median (95% range)	
Age at visit, months	24.8 (23.4-28.1)
Weight, kg	12.8 (10.2-16.1)
Peak weight velocity, mean (SD), kg/year	12.1 (2.1)
Body mass index at adiposity peak, mean (SD), kg/m^2	17.6 (0.8)
Age at adiposity peak, median (95% range), months	8.4 (7.8-9.6)
Child characteristics	
Age at MRI, mean (SD), years	10.1 (0.6)
Head circumference, mean (SD), cm	53.0 (1.6)
Length, mean (SD), cm	141.6 (6.6)
Weight, median (95% range), kg	33.8 (24.4-53.0)
Body mass index, median (95% range), kg/m^2	16.9 (14.1-24.3)

[1]Values are means (standard deviation), medians (95% range) or numbers of subjects (valid %).

Critical periods

Table 3 shows that increased weight gain during all of the examined time periods was associated with larger head circumference and total brain volume at 10 years of age. One SDS higher weight gain until the 2nd (5.7 cm^3; 95%CI, 1.2-10.2 cm^3) and 3rd trimester (15.3 cm^3; 95%CI, 11.0-19.6 cm^3), birth (20.8 cm^3; 95%CI, 16.4-25.1 cm^3), 6 months (15.6 cm^3; 95%CI, 11.2-19.9 cm^3), 12 months (11.3 cm^3; 95%CI, 7.0-15.6 cm^3), and 24 months (11.1 cm^3; 95%CI, 6.8-15.4 cm^3) was, independent of weights at the other age windows, associated with larger total brain volume. Similarly, higher weight gain during all of the examined time periods was associated with larger subcortical gray, cerebral and cerebellar gray and white matter volumes. After additional adjustment for childhood BMI, results remained significant (**Table S5** in Supplementary Materials). Post-hoc analyses showed that increased weight gain at birth and 6 months was associated with larger thalamus, putamen and pallidum volumes (**Table S6** in Supplementary Materials).

Longitudinal fetal and infant growth patterns

Compared with children with normal fetal and infant growth those with fetal weight deceleration followed by infant weight deceleration had the smallest total brain volume (32.5 cm^3; 95%CI, –53.2 to –11.9 cm^3), whereas those with fetal weight deceleration followed by infant weight acceleration had similar brain volumes. The largest brain volumes were observed for children who had both fetal and infant growth acceleration (**Table 4**). After additional adjustment for childhood BMI, results remained significant (**Table S7** in Supplementary Materials). Post-hoc analyses did not show significant associations for subcortical structures (**Table S8** in Supplementary Materials).

Table 2. Associations of birth outcomes with childhood brain outcomes[1]

Birth outcomes	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Gestational age, week	0.1 (0.0 to 0.1)**	4.7 (2.9 to 6.5)**	2.6 (1.7 to 3.5)**	1.2 (0.4 to 2.1)**	0.4 (0.2 to 0.6)**	0.2 (0.1 to 0.2)**	0.3 (0.2 to 0.4)**
< 37 weeks (N=138)	-0.3 (-0.5 to -0.0)*	-28.4 (-43.9 to -12.8)**	-16.6 (-24.3 to -8.9)**	-6.5 (-13.7 to 0.7)	-2.8 (-4.4 to -1.1)**	-0.8 (-1.3 to -0.4)**	-1.5 (-2.2 to -0.8)**
37-42 weeks (N=2,718)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>42 weeks (N=223)	0.3 (0.1 to 0.5)**	10.8 (-1.6 to 23.3)	7.1 (0.9 to 13.3)*	1.6 (-4.2 to 7.3)	0.8 (-0.5 to 2.1)	0.3 (-0.0 to 0.7)	0.9 (0.4 to 1.5)**
Birth weight, 500 g	0.3 (0.3 to 0.4)**	20.3 (17.3 to 23.2)**	9.8 (8.4 to 11.3)**	7.8 (6.4 to 9.1)**	1.4 (1.0 to 1.7)**	0.5 (0.4 to 0.6)**	0.8 (0.7 to 1.0)**
<2500 g (N=126)	-0.7 (-1.0 to -0.4)**	-44.9 (-61.1 to -28.7)**	-21.0 (-29.0 to -13.0)**	-16.4 (-23.9 to -8.9)**	-3.6 (-5.3 to -1.8)**	-1.6 (-2.1 to -1.1)**	-2.2 (-2.9 to -1.5)**
2500-4500 g (N=2,890)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4500 g (N=78)	0.7 (0.3 to 1.0)**	39.5 (19.1 to 60.0)**	18.7 (8.6 to 28.8)**	17.2 (7.8 to 26.6)**	1.5 (-0.7 to 3.7)	0.5 (-0.1 to 1.2)	1.6 (0.6 to 2.5)**
Size for gestational age, SDS	0.4 (0.3 to 0.4)**	21.9 (18.7 to 25.1)**	10.2 (8.6 to 11.8)**	9.0 (7.6 to 10.5)**	1.4 (1.0 to 1.7)**	0.4 (0.3 to 0.5)**	0.8 (0.7 to 10.0)**
Small (<10 percentile) (N=307)	-0.6 (-0.8 to -0.5)**	-36.6 (-47.4 to -25.9)**	-16.7 (-22.0 to -11.3)**	-15.5 (-20.5 to -10.5)**	-2.2 (-3.4 to -1.1)**	-0.8 (-1.1 to -0.5)**	-1.4 (-1.8 to -0.9)**
Appropriate (10-90 percentile) (N=2,458)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>90 percentile) (N=307)	0.6 (0.4 to 0.8)**	34.4 (23.6 to 45.1)**	14.8 (9.4 to 20.1)**	15.3 (10.3 to 20.3)**	2.2 (1.1 to 3.4)**	0.7 (0.4 to 1.0)**	1.3 (0.9 to 1.8)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for birth outcomes. Models are adjusted for child sex and age at the neuroimaging assessment, family income, and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *p < 0.05. ** p < 0.01.

Table 3. Critical periods during fetal and infant life and childhood brain outcomes[1]

Fetal and Infant Weight Standard Deviation Scores	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
At 20 weeks	0.1 (0.0 to 0.2)**	5.7 (1.2 to 10.2)*	2.9 (0.6 to 5.1)*	2.2 (0.1 to 4.3)*	0.3 (-0.2 to 0.8)	0.1 (-0.1 to 0.2)	0.2 (0.0 to 0.4)*
At 30 weeks	0.3 (0.2 to 0.3)**	15.3 (11.0 to 19.6)**	7.3 (5.1 to 9.4)**	6.1 (4.1 to 8.1)**	1.0 (0.5 to 1.5)**	0.4 (0.2 to 0.5)**	0.5 (0.3 to 0.7)**
At birth	0.4 (0.3 to 0.4)**	20.8 (16.4 to 25.1)**	9.5 (7.3 to 11.7)**	8.7 (6.6 to 10.7)**	1.3 (0.8 to 1.8)**	0.4 (0.3 to 0.6)**	0.8 (0.6 to 1.0)**
At 6 months	0.4 (0.3 to 0.4)**	15.6 (11.2 to 19.9)**	6.8 (4.7 to 9.0)**	6.1 (4.1 to 8.1)**	1.4 (1.0 to 1.9)**	0.4 (0.3 to 0.5)**	0.7 (0.5 to 0.9)**
At 12 months	0.3 (0.3 to 0.4)**	11.3 (7.0 to 15.6)**	4.2 (2.1 to 6.4)**	4.9 (2.8 to 7.0)**	1.3 (0.8 to 1.8)**	0.4 (0.3 to 0.6)**	0.4 (0.2 to 0.6)**
At 24 months	0.3 (0.2 to 0.4)**	11.1 (6.8 to 15.4)**	6.0 (3.9 to 8.1)**	3.7 (1.7 to 5.8)**	0.7 (0.2 to 1.2)**	0.3 (0.2 to 0.4)**	0.4 (0.2 to 0.6)**

[1]Values are linear regression coefficients (95% confidence intervals) from conditional analyses and reflect the change in cm of childhood head circumference (N=1,482) and in cm^3 of childhood brain structures (N=1,488) for fetal and infant weight. Models are adjusted for child sex and age at the neuroimaging assessment, family income, and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table 4. Associations of longitudinal fetal and infant growth patterns with childhood brain outcomes[1]

Growth patterns	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Fetal growth deceleration							
Infant growth deceleration (N= 80)	-0.8 (-1.2 to -0.5)**	-32.5 (-53.2 to -11.9)**	-16.5 (-26.8 to -6.2)**	-10.4 (-20.0 to -0.8)*	-3.8 (-6.0 to -1.6)**	-0.8 (-1.5 to -0.2)*	-0.8 (-1.8 to 0.1)
Infant normal growth (N= 245)	-0.3 (-0.5 to -0.1)*	-8.9 (-22.2 to 4.5)	-4.4 (-11.0 to 2.3)	-2.6 (-8.8 to 3.6)	-1.1 (-2.5 to 0.3)	-0.4 (-0.8 to 0.1)	-0.4 (-1.0 to 0.2)
Infant growth acceleration (N= 265)	0.1 (-0.1 to 0.3)	-5.0 (-18.0 to 8.0)	-2.2 (-8.7 to 4.3)	-3.2 (-9.3 to 2.9)	0.5 (-0.9 to 1.9)	0.2 (-0.2 to 0.6)	-0.3 (-0.9 to 0.3)
Fetal normal growth							
Infant growth deceleration (N= 210)	-0.2 (-0.4 to 0.1)	-5.6 (-19.7 to 8.4)	-0.5 (-7.5 to 6.5)	-3.3 (-9.8 to 3.3)	-1.2 (-2.7 to 0.3)	-0.4 (-0.8 to 0.1)	-0.3 (-0.9 to 0.4)
Infant normal growth (N= 532)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration (N= 274)	0.5 (0.3 to 0.7)**	14.6 (1.7 to 27.5)*	6.6 (0.2 to 13.0)*	6.2 (0.2 to 12.2)*	0.7 (-0.7 to 2.1)	0.5 (0.1 to 0.8)*	0.7 (0.1 to 1.2)*
Fetal growth acceleration							
Infant growth deceleration (N= 304)	0.1 (-0.1 to 0.3)	19.2 (6.7 to 31.6)**	9.1 (2.9 to 15.3)**	7.9 (2.1 to 13.6)**	1.2 (-0.1 to 2.5)	0.4 (0.1 to 0.8)*	0.5 (-0.0 to 1.1)
Infant normal growth (N= 313)	0.5 (0.3 to 0.7)**	30.6 (18.3 to 42.9)**	15.2 (9.1 to 21.4)**	12.3 (6.5 to 18.0)**	1.2 (-0.1 to 2.6)*	0.6 (0.3 to 1.0)**	1.2 (0.6 to 1.7)**
Infant growth acceleration (N= 94)	1.3 (0.9 to 1.6)**	44.7 (25.3 to 64.1)**	19.8 (10.2 to 29.5)**	20.9 (11.9 to 29.9)**	1.3 (-0.8 to 3.4)	0.9 (0.3 to 1.5)**	1.7 (0.8 to 2.6)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the difference in cm of childhood head circumference and in cm^3 for each childhood brain structures compared to children with normal fetal and infant growth. Models are adjusted for child sex and age at the neuroimaging assessment, family income, and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Infant growth

Table 5 shows that higher peak weight velocity and BMI at adiposity peak were associated with larger total brain volume (7.5 cm^3; 95%CI, 5.6-9.3 cm^3 and 19.3 cm^3; 95%CI, 14.6-23.9 cm^3, respectively), whereas age at adiposity peak was not. Similar results were observed for subcortical gray, cerebral and cerebellar gray and white matter volumes. Results remained significant after additional adjustment for childhood BMI (**Table S9** in Supplementary Materials). Post-hoc analyses showed that higher BMI at adiposity peak was associated with larger putamen volume (**Table S10** in Supplementary Materials).

All basic models are presented in **Table S11-S14** in Supplementary Materials.

DISCUSSION

In this population-based prospective cohort study, higher gestational age and birth weight were associated with larger brain volume at 10 years of age, suggesting that both fetal life and infancy seem to be independent critical periods for childhood neurodevelopment. Our findings indicate that compared with children with normal fetal and infant growth, those with fetal weight deceleration followed by infant catch-up growth had similar brain volume at 10 years of age.

Interpretation of main findings

The time from conception through 2 years of age, have been recognized as critical period of development.[1, 19] Poor growth, both in utero and in infancy, seems to influence the risk of adverse neurodevelopment outcomes later in life.[2, 14-17]

Gestational age and weight at birth have been largely used as determinants of child health. Previous studies have focused on infants born preterm or with low birth weight and have shown overall and regional reductions in brain volumes during childhood and adolescence.[10-12] Recent results from our own research group suggest an association of higher gestational age at birth, even within the term range, with larger brain volumes in 10-year-old children.[13] Our current findings show positive associations of gestational age, weight and size for gestational age at birth with childhood head circumference and total, cerebral and cerebellar gray and white matter volumes. Previous studies showed that birth weight in the upper growth centiles is also associated with both short- and long-term adverse neurodevelopment outcomes.[48, 49] We could not detect non-linear associations in our study. Further studies in clinical populations are needed to identify the specific optimal ranges for fetal and infant growth.

As fetal and infant growth are correlated with each other, it is important to study their independent associations with childhood brain morphology. We observed that faster weight

Table 5. Associations of infant growth patterns with childhood brain outcomes[1]

Characteristic	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Peak weight velocity, kg/y (N=2,654)	0.2 (0.2 to 0.2)**	7.5 (5.6 to 9.3)**	3.0 (2.1 to 3.9)**	3.2 (2.3 to 4.0)**	0.7 (0.5 to 0.9)**	0.2 (0.2 to 0.3)**	0.3 (0.2 to 0.4)**
BMI at adiposity peak, kg/m^2 (N=2,489)	0.5 (0.4 to 0.6)**	19.3 (14.6 to 23.9)**	8.7 (6.3 to 11.0)**	7.5 (5.4 to 9.7)**	1.5 (1.0 to 2.0)**	0.6 (0.5 to 0.7)**	0.8 (0.6 to 1.1)**
Age at adiposity peak, months (N=2,489)	0.1 (0.0 to 0.2)*	1.5 (-5.4 to 8.4)	0.8 (-2.6 to 4.2)	0.9 (-2.3 to 4.1)	-0.1 (-0.8 to 0.7)	-0.0 (-0.2 to 0.2)	-0.1 (-0.5 to 0.2)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for peak weight velocity, BMI and age at adiposity peak. Models are adjusted for child sex and age at the neuroimaging assessment, family income, and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

gain during each of the studied periods between mid-gestation and 24 months of age was associated with larger brain volumes independently of growth during any other age windows. The largest differences in brain volume were observed in relation to weight gain from late gestation to birth, whereas effect estimates were smallest in magnitude for growth after 6 months of age. This finding is in line with previous evidence that late gestation and the first postnatal months represent a critical period of brain development, during which the human brain experiences a striking growth spurt.[50]

To the best of our knowledge, the current study is the first to examine the associations of prospectively assessed fetal and infant growth patterns with childhood brain morphology measured by MRI. As compared with children with normal fetal and infant growth, those who experienced fetal weight deceleration but showed postnatal catch-up growth had similar brain volumes at 10 years. In contrast, a previous cross-sectional study in the Netherlands comparing brain morphology in children born SGA (N=36) with those born AGA (N=19) suggested that being born SGA, even after postnatal catch-up growth, was associated with reduced cerebral and cerebellar white matter volumes at the age of 4 to 7 years.[51] We also observed that higher peak weight velocity and BMI at adiposity peak were, independent of childhood BMI, associated with larger brain volumes. A previous study among 613 infants born preterm reported that greater infant weight and BMI gain to term were associated with better cognitive functioning.[24] A Finnish cohort of children born very preterm and with very low birth weight found that faster infant weight gain and head growth were associated with higher IQ among AGA children.[23] Altogether, these findings suggest that faster growth during the first 2 years of life might be associated with improved neurodevelopment outcomes later in life. As the current study focused on brain volumes only, caution is warranted regarding potential functional implications of the observed associations.

Fetal programming mechanisms might partly explain these associations. Maternal age, substance use, poor diet and psychological distress are known to influence fetal growth. An adverse intrauterine environmental may lead to adaptive fetal responses, i.e. circulatory redistribution, and negatively affect brain development.[52] Such stress-related factors may persist after pregnancy and alter infant brain growth. The current study suggests that suboptimal fetal or infant growth may be associated with fetal programming differences in brain outcomes on the longer term.[53] Another potential mechanism is the genetic predisposition.[54] Previous studies have found that the genetic background of birth weight is also related with head circumference at birth and in later life.[55, 56]

Altogether, the findings of the present study suggest that fetal and infant weight growth are associated with brain morphology in school-age children. Even though the observed effects are relatively small, they are important from a developmental and preventive perspective. Early postnatal life seems to be a window of opportunity to improve childhood growth and evaluate the healthy consequences for brain development. Future research should inves-

tigate potential causal pathways and underlying mechanisms of the observed associations and explore whether these associations link to neurocognitive outcomes.

Study Limitations

This study has some limitations. Because mothers of children with and without good quality of brain MRI data available were different regarding the socioeconomic and lifestyle characteristics, we cannot exclude the possibility of selection bias. We had a relatively healthy population. Children born preterm or SGA do not reflect the full neonatal group, but mainly late preterm born after 35 weeks, or children with mild SGA. Also, fetal weight estimated by ultrasound might be prone to measurement error, mainly among extremes (i.e. fetuses with low or high weight), which might have influenced our findings.[57] Further, for the infant weight change analysis we considered the period from birth to 24 months. When weight was missing at 24 months, we used weight at 12 or 6 months. We used all data available to optimize statistical power, but might have introduced bias. Brain morphology has been associated with behavioral and cognitive functions [58]. Future studies should investigate repeated assessments of brain morphology, as well as multi-modal neuroimaging in combination with behavioral and cognitive functioning in large population-based cohorts. Finally, owing to the observational design of the study, unmeasured residual confounding might still be present.

CONCLUSIONS

The results of this cohort study suggest that early-life growth is associated with brain morphology later in childhood. Both fetal life and infancy seem to be important critical periods of childhood brain development. Also, faster infant growth in the first 2 years of life may be associated with improved brain development later in life. Further studies are needed to assess whether a causal relationship exists and focus on potential long-term consequences of early growth patterns on brain structure and function.

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SUPPLEMENTARY MATERIALS

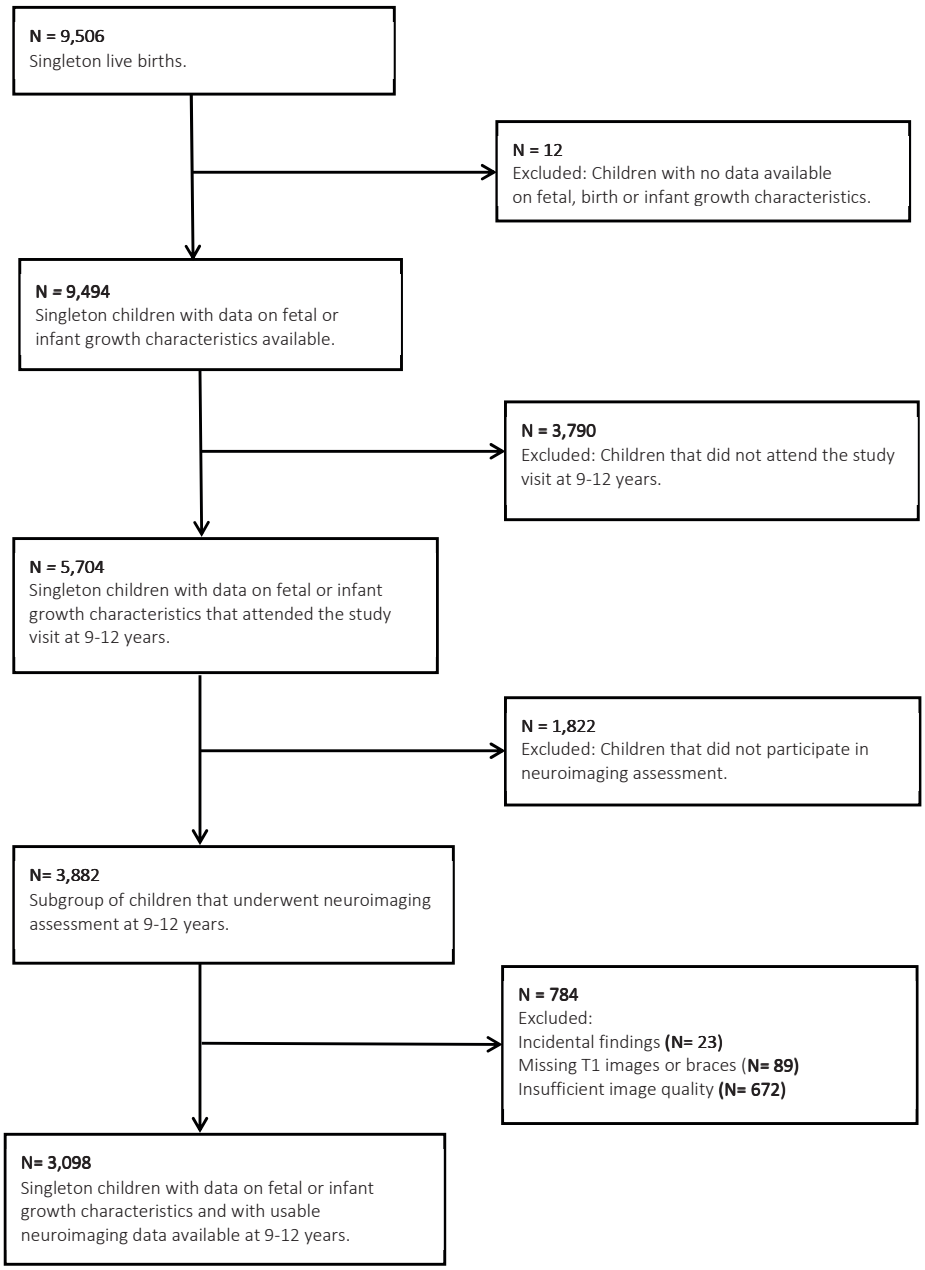


Figure S1. Flowchart of study population.

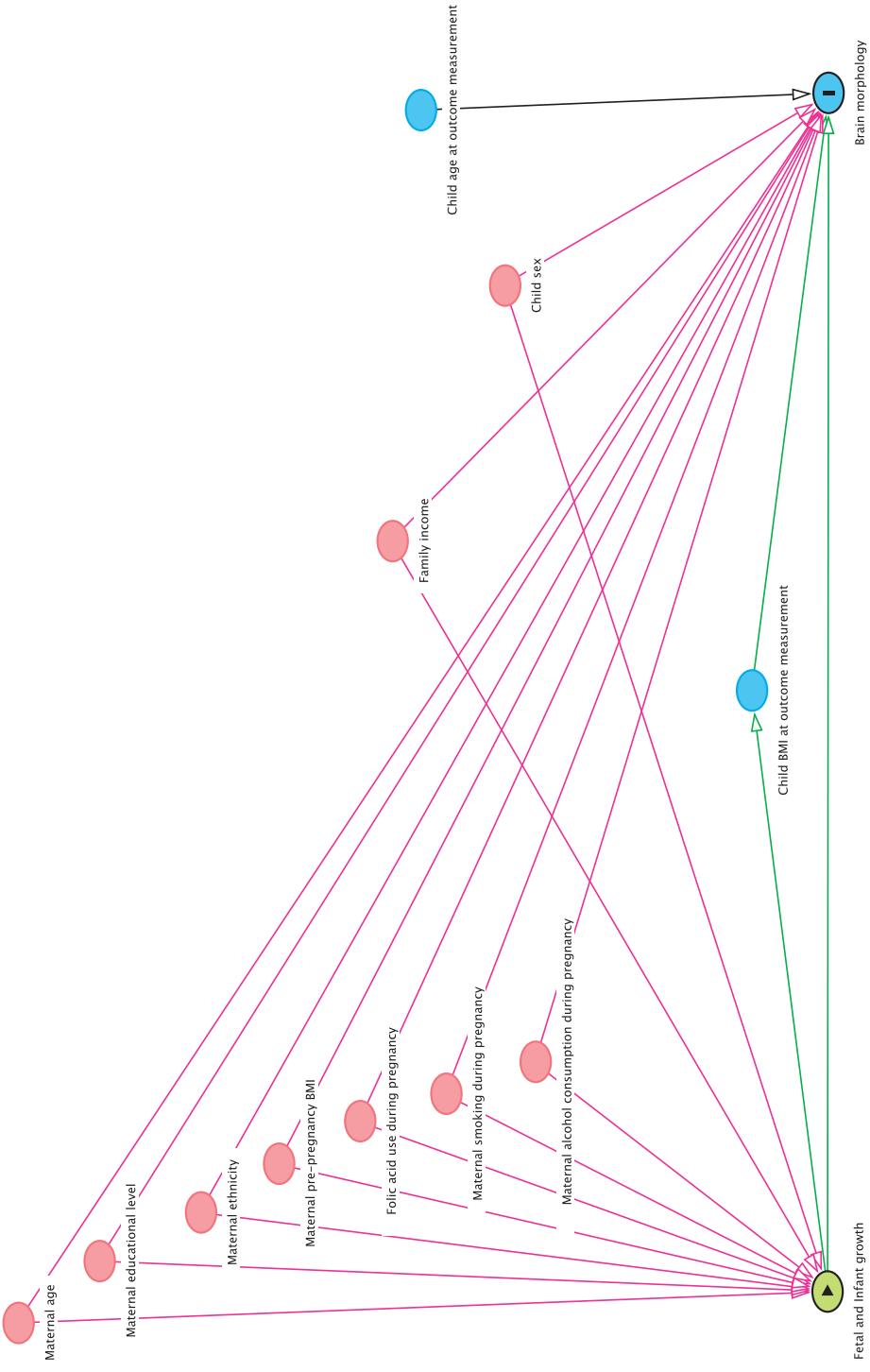


Figure S2. Directed Acyclic Graph showing the hypothesized relationship between childhood fetal and infant growth and brain outcomes and the covariates

Table S1. Fetal and infant growth measures

Fetal and Infant growth	Age of assessment	Technique/ information source	Outcome/measure	References
Fetal growth measures				
Gestational age	1st trimester	Ultrasound.	Continuous and categorized into preterm (<37 weeks), term (37-42 weeks) or post-term (>42 weeks).	Tunon K, et al. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol. 1996;8(3):178-85.
Head circumference Abdominal circumference Femur length	2nd and 3rd trimester	Ultrasound.	Used to estimate fetal weight.	Kooijman MN, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016 ;31(12):1243-64.
Estimated fetal weight	2nd and 3rd trimester	Calculated using the Hadlock formula.	Gestational-age-adjusted SDS for weight calculated using reference growth curves derived from the same cohort as the current study.	Hadlock FP,et al. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985;151(3):333-7. Verburg BO,et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.
Infant growth measures				
Weight	Birth	Medical records.	Sex- and gestational age-adjusted SDS for weight calculated based on North European reference charts.	Niklasson A, et al. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand. 1991;80(8-9):756-62.
	6, 12 and 24 months	Community health centers.	Sex- and age-adjusted SDS for weight calculated based on Dutch reference growth charts.	Fredriks AM, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res. 2000;47(3):316-23.

Table S1. Fetal and infant growth measures (*continued*)

Fetal and Infant growth	Age of assessment	Technique/ information source	Outcome/measure	References
Peak weight velocity	From birth to 36 months	Derived using the Reed1 model by sex on all weight measures.	Reflects the greatest weight growth in infancy.	Berkey CS, Reed RB. A model for describing normal and abnormal growth in early childhood. Hum Biol. 1987;59(6):973-87.
BMI at adiposity peak Age at adiposity peak	From 14 days to 18 months	Cubic mixed-effects model was fitted on log (BMI), adjusted for sex.	BMI was derived for each individual and the point where the curve reaches its maximum gives BMI and age at adiposity peak.	Sovio U, et al. Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. PLoS Genet. 2009;5(3):e1000409.
Fetal and Infant growth patterns				
Growth deceleration, normal growth or growth acceleration	From 2nd trimester to birth	Fetal weight change was the growth between the 2nd trimester and birth. Infant weight change was the growth from birth to 24 months. Growth acceleration or deceleration were defined as a change greater than 0.67 SD between time points.	Fetal and infant weight change were categorized into 3 groups (growth deceleration, normal growth, and growth acceleration), and combined variables that reflect 9 different growth patterns were created.	Ong KK, et al. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967-71.

Table S2. Comparison of characteristics between responders and non-responders[1]

Characteristics	Responders (n= 3,098)	Non-Responders (n= 2,606)	P Value[2]
Maternal characteristics			
Age at intake, mean (SD), years	31.1 (4.9)	30.8 (5.1)	< 0.05
Ethnicity, N(%)			< 0.05
Dutch	1,753 (57.8)	1,422 (56.4)	
Non-Dutch, Western	253 (8.3)	204 (8.1)	
Non-Dutch, Non-Western	1,026 (33.8)	895 (35.5)	
Prepregnancy body mass index, median (95% range), kg/m2	22.5 (18.0-34.8)	22.7 (18.2-34.3)	0.10
Education, N(%)			< 0.001
Primary school	186 (6.5)	220 (9.2)	
Secondary school	1,156 (40.6)	1,051 (44.0)	
Higher education	1,506 (52.9)	1,118 (46.8)	
Monthly household income, US$			< 0.001
< 1200	330 (13.7)	324 (16.1)	
1200-2000	380 (15.8)	381 (19.0)	
> 2000	1,699 (70.5)	1,304 (64.9)	
Folic acid used, N(%)			< 0.001
Yes	1,737 (79.9)	1,334 (74.5)	
Alcohol consumption, N (%)			< 0.05
Yes	1,238 (50.6)	992 (47.6)	
Smoking, N (%)			
Yes	501 (20.4)	477 (23.0)	< 0.05
Fetal characteristics			
Second trimester, median (95% range)			
Gestational age, weeks	20.5 (18.7-23.3)	20.6 (18.5-23.5)	< 0.05
Estimated fetal weight, g	362 (248-612)	367 (244-640)	< 0.05
Third trimester, median (95% range)			
Gestational age, weeks	30.4 (28.5-32.7)	30.4 (28.4-33.2)	0.11
Estimated fetal weight, g	1602 (1186-2150)	1601 (1171-2295)	0.20
Birth characteristics			
Gestational age at birth, median (95% range), weeks	40.1 (36.0-42.3)	40.1 (35.6-42.3)	0.05
Sex, N (%)			0.89
Girls	1557 (50.3)	1305 (50.1)	
Birth weight, mean (SD), g	3446 (553)	3440 (554)	0.06
Infant characteristics			
At 6 months, median (95% range)			
Age at visit, months	6.2 (5.2-7.9)	6.2 (5.3-8.9)	0.35
Weight, kg	7.8 (6.2-9.7)	7.8 (6.2-9.8)	0.35
At 12 months, median (95% range)			

Table S2. Comparison of characteristics between responders and non-responders[1] (*continued*)

Characteristics	Responders (n= 3,098)	Non-Responders (n= 2,606)	P Value[2]
Age at visit, months	11.0 (10.1-13.0)	11.1 (10.2-12.5)	< 0.001
Weight, kg	9.6 (7.7-11.8)	9.6 (7.6-12.0)	0.77
At 24 months, median (95% range)			
Age at visit, months	24.8 (23.4-28.1)	24.9 (23.4-28.3)	< 0.05
Weight, kg	12.8 (10.2-16.1)	12.9 (10.3-16.1)	0.55
Peak weight velocity, mean (SD), kg/y	12.1 (2.1)	12.2 (2.1)	0.62
Body mass index at adiposity peak, mean (SD), kg/m^2	17.6 (0.8)	17.6 (0.8)	0.48
Age at adiposity peak, median (95% range), months	8.4 (7.8-9.6)	8.4 (7.8-9.6)	0.14
Child characteristics			
Age at MRI, mean (SD), years	10.1 (0.6)	10.2 (0.8)	< 0.05
Length, mean (SD), cm	141.6 (6.6)	141.5 (6.8)	0.43
Weight, median (95% range), kg	33.8 (24.4-53.0)	34.0 (26.4-51.0)	0.37
Body mass index, median (95% range), kg/m^2	16.9 (14.1-24.3)	17.0 (14.3-23.5)	0.10

[1] Values are means (standard deviation), medians (95% range) or numbers of subjects (valid %).

[2] P-values for differences in subject characteristics between groups were calculated performing independent sample t-tests for normally distributed continuous variables, Mann-Whitney test for not normally distributed continuous variables and chi-square tests for categorical variables.

Table S3. Associations of birth outcomes with childhood brain outcomes (BMI models)[1]

Birth outcomes	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Gestational age, week	0.1 (0.0 to 0.1)**	4.8 (3.0 to 6.6)**	2.7 (1.8 to 3.6)**	1.3 (0.4 to 2.1)**	0.4 (0.2 to 0.6)**	0.1 (0.1 to 0.2)**	0.3 (0.2 to 0.4)**
< 37 weeks (N=138)	-0.2 (-0.5 to 0.0)	-29.0 (-44.5 to -13.4)**	-17.1 (-24.8 to -9.4)**	-6.6 (-13.8 to 0.5)	-2.8 (-4.5 to -1.2)**	-0.8 (-1.2 to -0.3)**	-1.5 (-2.2 to -0.8)**
37-41 weeks (N=2,718)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>42 weeks (N=223)	0.3 (0.1 to 0.5)**	11.5 (-1.0 to 24.0)	7.4 (1.2 to 13.5)*	2.0 (-3.8 to 7.7)	0.8 (-0.5 to 2.2)	0.4 (-0.0 to 0.7)	1.0 (0.4 to 1.5)**
Birth weight, 500g	0.3 (0.2 to 0.3)**	20.0 (17.0 to 22.9)**	9.7 (8.3 to 11.2)**	7.6 (6.2 to 9.0)**	1.4 (1.1 to 1.7)**	0.4 (0.3 to 0.5)**	0.8 (0.7 to 1.0)**
<2500 g (N=126)	-0.6 (-0.9 to -0.3)**	-44.3 (-60.5 to -28.1)**	-20.9 (-28.9 to -12.9)**	-16.1 (-23.5 to -8.6)**	-3.6 (-5.3 to -1.9)**	-1.5 (-2.0 to -1.0)**	-2.1 (-2.9 to -1.4)**
2500-4500 g (N=2,890)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4500 g (N=78)	0.6 (0.3 to 0.9)**	38.5 (18.1 to 58.9)**	18.3 (8.1 to 28.4)**	16.8 (7.4 to 26.2)**	1.5 (-0.7 to 3.7)	0.4 (-0.2 to 1.0)	1.5 (0.6 to 2.4)**
Size for gestational age, SDS	0.3 (0.2 to 0.3)**	21.5 (18.3 to 24.7)**	10.0 (8.4 to 11.6)**	8.9 (7.4 to 10.4)**	1.4 (1.1 to 1.8)**	0.4 (0.3 to 0.5)**	0.8 (0.6 to 0.9)**
Small (<10 percentile) (N=307)	-0.6 (-0.7 to -0.4)**	-36.1 (-46.8 to -25.3)**	-16.6 (-21.9 to -11.2)**	-15.2 (-20.1 to -10.2)**	-2.3 (-3.5 to -1.2)**	-0.7 (-1.0 to -0.4)**	-1.3 (-1.8 to -0.8)**
Appropriate (10-90 percentile) (N=2,458)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>90 percentile) (N=307)	0.5 (0.3 to 0.6)**	33.0 (22.2 to 43.8)**	14.2 (8.8 to 19.5)**	14.8 (9.8 to 19.8)**	2.2 (1.0 to 3.4)**	0.6 (0.2 to 0.9)**	1.3 (0.8 to 1.8)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for birth outcomes. Models are adjusted for child sex, BMI and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *p < 0.05. ** p < 0.01.

Table S4. Associations of birth outcomes with childhood brain outcomes (Subcortical structures)[1]

Birth outcomes	Difference (95% Confidence Interval)						
	Thalamus volume (cm^3)	Amygdala volume (cm^3)	Hippocampus volume (cm^3)	Globus Pallidus volume (cm^3)	Putamen volume (cm^3)	Caudate nucleus volume (cm^3)	Nucleus accumbens volume (cm^3)
Gestational age, week	0.07 (0.05 to 0.08)**	0.00 (-0.01 to 0.01)	0.01 (-0.01 to 0.02)	0.02 (0.01 to 0.03)**	0.03 (0.01 to 0.05)**	0.02 (0.00 to 0.03)*	0.00 (-0.00 to 0.01)
Birth weight, 500g	0.09 (0.06 to 0.12)**	-0.01 (-0.02 to -0.00)*	-0.00 (-0.03 to 0.01)	0.03 (0.02 to 0.04)**	0.05 (0.01 to 0.08)**	0.02 (-0.00 to 0.05)	0.01 (00.0 to 0.02)**
Size for gestational age, SDS	0.03 (-0.00 to 0.06)	-0.02 (-0.03 to -0.01)**	-0.02 (-0.04 to 0.00)	0.02 (0.01 to 0.03)**	0.02 (-0.02 to 0.06)	0.01 (-0.02 to 0.04)	0.01 (0.00 to 0.02)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for birth outcomes. Models are adjusted for child sex, intracranial volume and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S5. Critical periods during fetal and infant life and childhood brain outcomes (BMI models)[1]

Fetal and Infant Weight Standard Deviation Scores	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
At 20 weeks	0.1 (0.0 to 0.2)**	5.6 (1.1 to 10.1)*	2.9 (0.7 to 5.2)*	2.1 (0.0 to 4.2)	0.4 (-0.1 to 0.9)	0.1 (-0.1 to 0.2)	0.2 (-0.0 to 0.4)
At 30 weeks	0.2 (0.2 to 0.3)**	15.9 (11.6 to 20.2)**	7.6 (5.5 to 9.8)**	6.3 (4.3 to 8.4)**	1.1 (0.6 to 1.5)**	0.3 (0.2 to 0.5)**	0.5 (0.3 to 0.7)**
At birth	0.3 (0.3 to 0.4)**	21.1 (16.8 to 25.5)**	9.7 (7.6 to 11.9)**	8.8 (6.7 to 10.9)**	1.4 (0.9 to 1.9)**	0.4 (0.2 to 0.5)**	0.8 (0.6 to 1.0)**
At 6 months	0.3 (0.2 to 0.3)**	16.4 (11.9 to 20.8)**	7.3 (5.1 to 9.6)**	6.4 (4.3 to 8.5)**	1.6 (1.1 to 2.1)**	0.3 (0.1 to 0.4)**	0.7 (0.5 to 0.9)**
At 12 months	0.3 (0.2 to 0.3)**	12.3 (7.9 to 16.7)**	4.8 (2.6 to 7.0)**	5.3 (3.2 to 7.4)**	1.4 (1.0 to 1.9)**	0.3 (0.2 to 0.5)**	0.4 (0.2 to 0.6)**
At 24 months	0.2 (0.1 to 0.3)**	11.8 (7.4 to 16.2)**	6.4 (4.2 to 8.6)**	4.0 (2.0 to 6.1)**	0.8 (0.3 to 1.3)**	0.2 (0.0 to 0.3)*	0.4 (0.2 to 0.6)**

[1]Values are linear regression coefficients (95% confidence intervals) from conditional analyses and reflect the change in cm of childhood head circumference (N=1,482) and in cm^3 of childhood brain structures (N=1,488) for fetal and infant weight. Models are adjusted for child sex, BMI and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S6. Critical periods during fetal and infant life and childhood brain outcomes (Subcortical structures)[1]

Fetal and Infant Weight Standard Deviation Scores	Difference (95% Confidence Interval)						
	Thalamus volume (cm^3)	Amygdala volume (cm^3)	Hippocampus volume (cm^3)	Globus Pallidus volume (cm^3)	Putamen volume (cm^3)	Caudate nucleus volume (cm^3)	Nucleus accumbens volume (cm^3)
At 20 weeks	0.01 (-0.04 to 0.05)	0.00 (-0.01 to 0.02)	0.00 (-0.03 to 0.03)	-0.01 (-0.03 to 0.01)	-0.02 (-0.07 to 0.03)	0.00 (-0.04 to 0.04)	0.00 (-0.01 to 0.01)
At 30 weeks	0.02 (-0.03 to 0.06)	-0.01 (-0.03 to 0.00)	0.01 (-0.02 to 0.04)	0.01 (-0.01 to 0.03)	0.01 (-0.04 to 0.06)	-0.01 (-0.05 to 0.03)	0.01 (0.00 to 0.02)*
At birth	0.08 (0.04 to 0.13)**	-0.01 (-0.03 to 0.00)	-0.00 (-0.03 to 0.03)	0.03 (0.01 to 0.04)**	0.04 (-0.01 to 0.09)	0.01 (-0.03 to 0.05)	0.01 (00.0 to 0.02)*
At 6 months	0.05 (0.01 to 0.09)*	0.01 (-0.00 to 0.03)	0.02 (-0.01 to 0.05)	0.02 (0.00 to 0.04)*	0.08 (0.03 to 0.13)**	0.03 (-0.02 to 0.07)	0.00 (-0.01 to 0.01)
At 12 months	-0.00 (-0.05 to 0.04)	0.01 (-0.01 to 0.02)	0.03 (-0.00 to 0.06)	-0.01 (-0.03 to 0.01)	-0.03 (-0.08 to 0.02)	0.01 (-0.03 to 0.05)	-0.00 (-0.01 to 0.01)
At 24 months	-0.01 (-0.06 to 0.03)	-0.01 (-0.02 to 0.01)	0.02 (-0.01 to 0.05)	-0.01 (-0.03 to 0.01)	-0.01 (-0.05 to 0.04)	-0.03 (-0.07 to 0.02)	-0.01 (-0.01 to 0.00)

[1]Values are linear regression coefficients (95% confidence intervals) from conditional analyses and reflect the change in cm of childhood head circumference (N=1,482) and in cm^3 of childhood brain structures (N=1,488) for fetal and infant weight. Models are adjusted for child sex, intracranial volume and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S7. Associations of longitudinal fetal and infant growth patterns with childhood brain outcomes (BMI models)[1]

Growth patterns	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Fetal growth deceleration							
Infant growth deceleration (N= 80)	-0.5 (-0.9 to -0.2)**	-28.6 (-49.4 to -7.8)**	-14.8 (-25.2 to -4.5)**	-8.9 (-18.6 to 0.8)	-3.6 (-5.9 to -1.4)**	-0.5 (-1.1 to 0.1)	-0.6 (-1.6 to 0.3)
Infant normal growth (N= 245)	-0.2 (-0.4 to 0.0)	-7.8 (-21.2 to 5.6)	-4.0 (-10.7 to 2.7)	-2.1 (-8.4 to 4.1)	-1.1 (-2.5 to 0.3)	-0.2 (-0.6 to 0.2)	-0.3 (-0.9 to 0.3)
Infant growth acceleration (N= 265)	0.0 (-0.2 to 0.2)	-6.4 (-19.5 to 6.6)	-2.8 (-9.4 to 3.7)	-3.7 (-9.8 to 2.4)	0.4 (-1.0 to 1.9)	0.0 (-0.4 to 0.4)	-0.4 (-0.9 to 0.2)
Fetal normal growth							
Infant growth deceleration (N= 210)	0.0 (-0.3 to 0.2)	-2.7 (-16.8 to 11.4)	0.7 (-6.3 to 7.7)	-2.1 (-8.6 to 4.5)	-1.1 (-2.6 to 0.4)	-0.2 (-0.6 to 0.3)	-0.1 (-0.7 to 0.5)
Infant normal growth (N= 532)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration (N= 274)	0.3 (0.1 to 0.5)**	12.7 (-0.3 to 25.7)	5.8 (-0.6 to 12.3)	5.5 (-0.5 to 11.6)	0.6 (-0.8 to 2.0)	0.2 (-0.2 to 0.6)	0.6 (-0.0 to 1.1)
Fetal growth acceleration							
Infant growth deceleration (N= 304)	0.2 (0.0 to 0.4)	20.7 (8.2 to 33.1)**	9.7 (3.5 to 15.9)**	8.5 (2.7 to 14.3)**	1.3 (-0.1 to 2.6)	0.5 (0.2 to 0.9)**	0.6 (0.1 to 1.2)*
Infant normal growth (N= 313)	0.4 (0.2 to 0.6)**	30.1 (17.7 to 42.5)**	15.2 (9.1 to 21.4)**	11.9 (6.1 to 17.7)**	1.4 (0.0 to 2.7)*	0.5 (0.1 to 0.8)*	1.1 (0.5 to 1.6)**
Infant growth acceleration (N= 94)	1.0 (0.7 to 1.3)**	39.9 (20.4 to 59.4)**	17.9 (8.1 to 27.6)**	18.9 (9.9 to 28.0)**	1.1 (-1.0 to 3.2)	0.5 (-0.1 to 1.1)	1.5 (0.6 to 2.3)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the difference in cm of childhood head circumference and in cm^3 for each childhood brain structures compared to children with normal fetal and infant growth. Models are adjusted for child sex, BMI and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S8. Associations of longitudinal fetal and infant growth patterns with childhood brain outcomes (Subcortical structures)[1]

Growth patterns	Difference (95% Confidence Interval)						
	Thalamus volume (cm^3)	Amygdala volume (cm^3)	Hippocampus volume (cm^3)	Globus Pallidus volume (cm^3)	Putamen volume (cm^3)	Caudate nucleus volume (cm^3)	Nucleus accumbens volume (cm^3)
Fetal growth acceleration							
Infant growth deceleration (N= 304)	-0.02 (-0.13 to 0.09)	-0.05 (-0.08 to -0.01)*	-0.01 (-0.08 to 0.06)	0.02 (-0.02 to 0.06)	0.08 (-0.04 to 0.19)	-0.04 (-0.13 to 0.06)	0.02 (0.00 to 0.04)*
Infant normal growth (N= 313)	0.03 (-0.08 to 0.13)	-0.00 (-0.04 to 0.03)	0.02 (-0.05 to 0.09)	0.01 (-0.03 to 0.05)	0.02 (-0.09 to 0.14)	0.05 (-0.05 to 0.14)	0.02 (-0.00 to 0.04)
Infant growth acceleration (N= 94)	-0.06 (-0.25 to 0.12)	-0.02 (-0.08 to 0.04)	0.01 (-0.11 to 0.13)	-0.04 (-0.11 to 0.03)	0.06 (-0.14 to 0.26)	-0.09 (-0.26 to 0.07)	-0.01 (-0.05 to 0.02)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the difference in cm of childhood head circumference and in cm^3 for each childhood brain structures compared to children with normal fetal and infant growth. Models are adjusted for child sex, intracranial volume and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S9. Associations of infant growth patterns with childhood brain outcomes (BMI models)[1]

Characteristic	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Peak weight velocity, kg/y (N=2,654)	0.1 (0.1 to 0.2)**	6.7 (4.7 to 8.6)**	2.7 (1.7 to 3.6)**	2.9 (2.0 to 3.7)**	0.7 (0.5 to 0.9)**	0.1 (0.1 to 0.2)**	0.3 (0.2 to 0.3)**
BMI at adiposity peak, kg/m^2 (N=2,489)	0.3 (0.2 to 0.4)**	17.7 (12.7 to 22.7)**	8.1 (5.6 to 10.6)**	6.8 (4.5 to 9.2)**	1.7 (1.1 to 2.2)**	0.3 (0.2 to 0.5)**	0.7 (0.5 to 1.0)**
Age at adiposity peak, months (N=2,489)	0.1 (-0.0 to 0.2)	0.8 (-6.1 to 7.7)	0.5 (-2.9 to 4.0)	0.6 (-2.6 to 3.8)	-0.1 (-0.8 to 0.7)	-0.1 (-0.3 to 0.1)	-0.2 (-0.5 to 0.1)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for peak weight velocity (PWV), body mass index at adiposity peak (BMIAP) and age at adiposity peak (AGEAP). Models are adjusted for child sex, BMI and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *p < 0.05. ** p < 0.01.

Table S10. Associations of infant growth patterns with childhood brain outcomes (Subcortical structures)[1]

Characteristic	Difference (95% Confidence Interval)						
	Thalamus volume (cm^3)	Amygdala volume (cm^3)	Hippocampus volume (cm^3)	Globus Pallidus volume (cm^3)	Putamen volume (cm^3)	Caudate nucleus volume (cm^3)	Nucleus accumbens volume (cm^3)
Peak weight velocity, kg/y (N=2,654)	0.01 (-0.01 to 0.03)	0.01 (-0.00 to 0.01)	0.01 (0.00 to 0.02)	0.00 (-0.01 to 0.01)	0.02 (-0.00 to 0.04)	-0.00 (-0.02 to 0.01)	-0.00 (-0.01 to 0.00)
BMI at adiposity peak, kg/m^2 (N=2,489)	0.05 (-0.00 to 0.09)	-0.00 (-0.02 to 0.2)	0.02 (-0.01 to 0.05)	0.02 (0.00 to 0.04)*	0.08 (0.03 to 0.13)**	0.03 (-0.01 to 0.07)	-0.00 (-0.01 to 0.01)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for peak weight velocity, BMI at adiposity peak and age at adiposity peak. Models are adjusted for child sex, intracranial volume and age at the neuroimaging assessment, family income and maternal age at intake, ethnicity, pre-pregnancy BMI, educational level, smoking, alcohol, and folic acid use during pregnancy. *$p < 0.05$. ** $p < 0.01$.

Table S11. Associations of birth outcomes with childhood brain outcomes (basic models)[1]

Birth outcomes	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Gestational age, week	0.1 (0.0 to 0.1)**	6.1 (4.2 to 8.0)**	3.3 (2.4 to 4.3)**	1.7 (0.9 to 2.6)**	0.5 (0.3 to 0.7)**	0.2 (0.1 to 0.2)**	0.3 (0.2 to 0.4)**
< 37 weeks (N=138)	-0.3 (-0.5 to -0.0)*	-35.4 (-51.6 to -19.1)**	-20.3 (-28.4 to -12.2)**	-8.8 (-16.1 to -1.4)*	-3.5 (-5.3 to -1.8)**	-0.8 (-1.3 to -0.4)**	-1.8 (-2.5 to -1.1)**
37-41 weeks (N=2,718)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>42 weeks (N=223)	0.3 (0.1 to 0.5)**	15.0 (2.0 to 28.0)*	9.1 (2.6 to 15.6)**	3.3 (-2.6 to 9.1)	1.0 (-0.3 to 2.5)	0.4 (0.0 to 0.8)*	1.1 (0.5 to 1.6)**
Birth weight, 500g	0.3 (0.3 to 0.4)**	24.3 (21.3 to 27.2)**	11.9 (10.4 to 13.4)**	9.1 (7.8 to 10.5)**	1.7 (1.4 to 2.0)**	0.5 (0.4 to 0.6)**	1.0 (0.8 to 1.1)**
<2500 g (N=126)	-0.7 (-1.0 to -0.5)**	-51.7 (-68.6 to -34.9)**	-24.4 (-32.8 to -16.0)**	-18.9 (-26.5 to -11.3)**	-4.2 (-6.0 to -2.4)**	-1.7 (-2.2 to -1.2)**	-2.4 (-3.2 to -1.7)**
2500-4500 g (N=2,890)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
>4500 g (N=78)	0.7 (0.4 to 1.0)**	43.6 (22.3 to 64.9)**	20.6 (9.9 to 31.2)**	19.0 (9.3 to 28.6)**	1.7 (-0.6 to 4.0)	0.6 (0.0 to 1.2)*	1.6 (0.7 to 2.6)**
Size for gestational age, SDS	0.4 (0.3 to 0.4)**	25.9 (22.7 to 29.1)**	12.2 (10.6 to 13.9)**	10.4 (9.0 to 11.9)**	1.8 (1.4 to 2.1)**	0.5 (0.4 to 0.6)**	0.9 (0.8 to 1.1)**
Small (<10 percentile) (N=307)	-0.7 (-0.9 to -0.5)**	-45.8 (-56.9 to -34.7)**	-21.4 (-26.9 to -15.9)**	-18.6 (-23.7 to -13.6)**	-3.1 (-4.3 to -1.9)**	-0.9 (-1.2 to -0.6)**	-1.6 (-2.1 to -1.2)**
Appropriate (10-90 percentile) (N=2,458)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Large (>90 percentile) (N=307)	0.7 (0.5 to 0.8)**	41.2 (30.1 to 52.3)**	18.2 (12.7 to 23.8)**	17.8 (12.8 to 22.8)**	2.8 (1.6 to 4.0)**	0.8 (0.5 to 1.1)**	1.5 (1.1 to 2.0)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for birth outcomes. Models are adjusted for child sex and age at the neuroimaging assessment.*p < 0.05. ** p < 0.01.

Table S12. Critical periods during fetal and infant life and childhood brain outcomes (basic models)[1]

Infant and Fetal Weight Standard Deviation Scores	Difference (95% CI)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
At 20 weeks	0.1 (0.0 to 0.2)**	6.0 (1.4 to 10.5)**	3.1 (0.8 to 5.4)**	2.3 (0.2 to 4.4)*	0.3 (-0.2 to 0.8)	0.1 (-0.1 to 0.2)	0.2 (-0.0 to 0.4)
At 30 weeks	0.3 (0.2 to 0.3)**	16.1 (11.8 to 20.5)**	7.7 (5.5 to 9.9)**	6.5 (4.5 to 8.5)**	1.0 (0.6 to 1.5)**	0.4 (0.3 to 0.5)**	0.5 (0.3 to 0.7)**
At birth	0.4 (0.3 to 0.4)**	23.2 (18.8 to 27.5)**	10.8 (8.6 to 13.0)**	9.4 (7.4 to 11.4)**	1.6 (1.1 to 2.1)**	0.4 (0.3 to 0.6)**	0.9 (0.7 to 1.1)**
At 6 months	0.4 (0.3 to 0.4)**	12.9 (8.5 to 17.2)**	5.2 (3.0 to 7.4)**	5.4 (3.4 to 7.4)**	1.1 (0.7 to 1.6)**	0.4 (0.3 to 0.5)**	0.7 (0.5 to 0.8)**
At 12 months	0.3 (0.3 to 0.4)**	12.3 (7.9 to 16.6)**	4.8 (2.6 to 7.0)**	5.3 (3.3 to 7.3)**	1.3 (0.9 to 1.8)**	0.4 (0.3 to 0.6)**	0.4 (0.2 to 0.6)**
At 24 months	0.3 (0.2 to 0.4)**	11.7 (7.3 to 16.0)**	6.3 (4.1 to 8.5)**	4.0 (2.0 to 6.0)**	0.7 (0.2 to 1.2)**	0.3 (0.2 to 0.4)**	0.4 (0.2 to 0.6)**

[1]Values are linear regression coefficients (95% confidence intervals) from conditional analyses and reflect the change in cm of childhood head circumference and in cm^3 of childhood brain structures for fetal and infant weight. Models are adjusted for child sex and age at the neuroimaging assessment. *$p < 0.05$. ** $p < 0.01$.

Table S13. Associations of longitudinal fetal and infant growth patterns with childhood brain outcomes (basic models)[1]

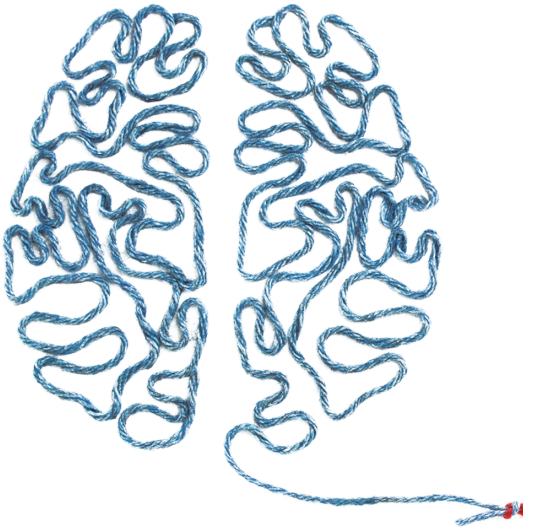
Growth patterns	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Fetal growth deceleration							
Infant growth deceleration (N= 80)	-0.8 (-1.2 to -0.5)**	-31.6 (-53.0 to -10.2)**	-15.9 (-26.6 to -5.2)**	-10.3 (-20.0 to -0.5)*	-3.6 (-5.9 to -1.3)**	-0.8 (-1.5 to -0.2)*	-0.8 (-1.8 to 0.1)
Infant normal growth (N= 245)	-0.3 (-0.5 to -0.1)*	-14.5 (-28.3 to -0.7)*	-7.2 (-14.1 to -0.3)*	-4.4 (-10.7 to 1.9)	-1.8 (-3.3 to -0.3)*	-0.4 (-0.8 to 0.0)	-0.6 (-1.2 to -0.0)*
Infant growth acceleration (N= 265)	0.1 (-0.1 to 0.3)	-11.3 (-24.7 to 2.1)	-5.7 (-12.4 to 1.1)	-5.0 (-11.2 to 1.1)	-0.3 (-1.8 to 1.2)	0.1 (-0.3 to 0.5)	-0.5 (-1.1 to 0.1)
Fetal normal growth							
Infant growth deceleration (N= 210)	-0.2 (-0.4 to 0.1)	-4.7 (-19.2 to 9.9)	0.0 (-7.3 to 7.3)	-2.9 (-9.5 to 3.8)	-1.3 (-2.8 to 0.3)	-0.3 (-0.8 to 0.1)	-0.2 (-0.9 to 0.4)
Infant normal growth (N= 532)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Infant growth acceleration (N= 274)	0.5 (0.3 to 0.7)**	7.3 (-6.0 to 20.6)	2.5 (-4.2 to 9.1)	4.2 (-1.9 to 10.3)	-0.2 (-1.7 to 1.2)	0.4 (0.0 to 0.8)*	0.4 (-0.2 to 1.0)
Fetal growth acceleration							
Infant growth deceleration (N= 304)	0.2 (-0.0 to 0.4)	22.7 (9.9 to 35.6)**	10.7 (4.3 to 17.1)**	9.3 (3.5 to 15.2)**	1.5 (0.1 to 2.9)*	0.5 (0.1 to 0.9)**	0.7 (0.1 to 1.2)*
Infant normal growth (N= 313)	0.6 (0.4 to 0.8)**	35.1 (22.3 to 47.8)**	17.5 (11.1 to 23.9)**	13.9 (8.1 to 19.7)**	1.6 (0.2 to 2.9)*	0.7 (0.3 to 1.1)**	1.3 (0.7 to 1.8)**
Infant growth acceleration (N= 94)	1.3 (0.9 to 1.6)**	35.9 (15.9 to 55.9)**	15.3 (5.3 to 25.3)**	18.2 (9.1 to 27.4)**	0.1 (-2.0 to 2.3)	0.9 (0.3 to 1.5)**	1.4 (0.5 to 2.2)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm of childhood head circumference and in cm^3 for each childhood brain structures compared to children with normal fetal and infant growth. Models are adjusted for child sex and age at the neuroimaging assessment.*p < 0.05. ** p < 0.01.

Table S14. Associations of infant growth patterns with childhood brain outcomes (basic models)[1]

Characteristic	Difference (95% Confidence Interval)						
	Head circumference (cm)	Total brain volume (cm^3)	Cerebral gray matter volume (cm^3)	Cerebral white matter volume (cm^3)	Cerebellar gray matter volume (cm^3)	Cerebellar white matter volume (cm^3)	Subcortical gray matter volume (cm^3)
Peak weight velocity, kg/y (N=2,654)	0.2 (0.2 to 0.2)**	4.5 (2.6 to 6.4)**	1.5 (0.5 to 2.4)**	2.2 (1.4 to 3.1)**	0.4 (0.2 to 0.6)**	0.2 (0.1 to 0.3)**	0.2 (0.1 to 0.3)**
BMI at adiposity peak, kg/m^2 (N=2,489)	0.5 (0.5 to 0.6)**	16.4 (11.6 to 21.2)**	7.1 (4.7 to 9.5)**	6.8 (4.6 to 9.0)**	1.1 (0.6 to 1.6)**	0.6 (0.5 to 0.7)**	0.7 (0.5 to 0.9)**
Age at adiposity peak, months (N=2,489)	0.1 (0.0 to 0.2)*	6.9 (-0.2 to 14.0)	3.7 (0.2 to 7.2)*	2.6 (-0.6 to 5.9)	0.5 (-0.2 to 1.3)	0.0 (-0.2 to 0.2)	0.0 (-0.3 to 0.3)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain structures for peak weight velocity, BMI and age at adiposity peak. Models are adjusted for child sex and age at the neuroimaging assessment. *$p < 0.05$. ** $p < 0.01$.



Chapter 3.3

Body fat, cardiovascular risk factors and brain structure in school-age children

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Adapted from: *Int J Obes (Lond). 2021;45(11):2425-31.*

ABSTRACT

Background: In adults, cardiovascular risk factors are known to be associated with brain health. We hypothesized that these associations are already present at school-age. We examined the associations of adverse body fat measures and cardiovascular risk factors with brain structure, including volumetric measures and white matter microstructure, in 10-year-old children.

Methods: We performed a cross-sectional analysis in a population-based prospective cohort study in Rotterdam, the Netherlands. Analyses were based on 3,098 children aged 10 years with neuroimaging data and at least one measurement of body fat and cardiovascular risk factors. Body fat measures included body mass index (BMI), fat mass index and android fat mass percentage obtained by Dual-energy X-ray absorptiometry. Cardiovascular risk factors included blood pressure, and serum glucose, insulin and lipids blood concentrations. Structural neuroimaging, including global and regional brain volumes, was quantified by magnetic resonance imaging. DTI was used to assess white matter microstructure, including global fractional anisotropy (FA) and mean diffusivity (MD).

Results: As compared to children with a normal weight, those with underweight had a smaller total brain and white matter volumes (differences -18.10 (95% Confidence Interval (CI) -30.97,-5.22) cm^3, -10.64 (95% CI -16.82,-4.47) cm^3, respectively). In contrast, one SDS (Standard Deviation Score) increase in fat mass index was associated with a smaller gray matter volume (differences -3.48 (95% CI -16.82,-4.47) cm^3). Also, one SDS increase in android fat mass percentage was associated with lower white matter diffusivity (difference -0.06 (95% CI -0.10,-0.02) SDS). None of the other cardiovascular risk factors were associated with any of the brain outcomes.

Conclusions: Body fat measures, but not other cardiovascular risk factors, were associated with structural neuroimaging outcomes in school-aged children. Prospective studies are needed to assess causality, direction and long-term consequences of the associations.

INTRODUCTION

Obesity and adverse cardiovascular risk profile among children are highly prevalent.[1] In the United States, approximately 20% of both children and adolescents have an adverse lipid profile, and more than 10% have either borderline high or high blood pressure.[2] Long-term exposure to adverse cardiovascular risk factors can affect the structure and function of cerebral blood vessels and leads to low-grade systemic inflammation affecting the brain.[3, 4] Results from longitudinal studies suggested that improving cardiovascular profile in young adults is associated with greater cerebral volumes, larger cerebral vessel caliber and fewer white matter hyperintensities later in life.[5, 6] In addition, results from a study using the UK Biobank data demonstrated an association of obesity with smaller subcortical gray matter volumes in adulthood.[7] In line with these results, a growing body of studies among children and adolescents suggest associations of higher body mass index (BMI) with smaller whole brain volumes, including regional gray matter volumes.[8-10] Obesity and adverse cardiovascular risk factors have been also associated with deficits in neurocognitive and executive functions in childhood and adolescence.[8-15] Recent results from our own research group, the Generation R Study, showed that higher blood pressure was associated with lower cognitive function in 6-year-old children.[15]. In addition, in a study that used gyrification of the brain as an outcome, demonstrated that not only higher BMI, but also lower BMI was associated with differences in brain morphology in children.[16] Previous studies propose that the associations with body fat measures, cardiovascular risk factors and brain morphology may already be apparent years before the related adult diseases develop.[17] However, studies performed thus far have mainly focused on indirect measurements of adiposity such as BMI and waist circumference. Also, a major literature gap is the lack of large population-based datasets evaluating the association of cardiovascular risk factors and brain morphology in children.

We hypothesized that an adverse body fat distribution or cardiovascular profile might be related with brain development already in childhood. In a population-based cohort study of 3,098 children aged 10 years, we examined the associations of body fat measures and cardiovascular risk factors, such as BMI, fat mass index, android fat mass percentage, blood pressure, and insulin, glucose, and lipids concentrations with brain structure. Outcomes measures included brain volumes and white matter microstructure.

METHODS

Study design

This cross-sectional analysis was embedded within the Generation R Study, a prospective population-based cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[18] The Medical Ethical Committee of the Erasmus Medical Center approved the study

protocols (MEC 198.782/2001/31). Written informed consent was obtained from the parents of all participating children. Children with a mean age of 9.8 years were invited to visit our research center at the Erasmus MC-Sophia Children's Hospital.[19, 20] A total of 5,706 singleton children visited the research center, and of these, 5,547 children had at least one measurement of body fat and cardiovascular risk factors. We excluded 1,664 children who did not participate in the neuroimaging visit and an additional 785 children due to insufficient quality of neuroimaging data. No information about specific reasons of declining participation was available. Analyses were based on 3,098 children with structural MRI and 2,961 children with Diffusion Tensor Imaging (DTI) outcomes (**Figure S1** in Supplementary Materials).

Body fat and cardiovascular risk factors

Body mass index (kg/m^2) was calculated using height and weight, both measured without heavy clothing and shoes. Age- and sex-adjusted standard deviation scores (SDS) for BMI were obtained with Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[21] BMI categories (underweight, normal weight, overweight/obesity) were based on the International Obesity Task Force cut-offs.[22] Total body fat mass was measured by Dual-energy X-ray absorptiometry (iDXA, GE140 Lunar, 2008, Madison, WI, USA, enCORE software v.12.6).[23] To obtain a fat mass index independent of height, we divided total fat mass by height^4 and estimated the optimal adjustment by log-log regression analyses.[24, 25] Android fat mass was computed as a percentage of total fat mass.[26] Blood pressure was measured at the right brachial artery four times with one-minute intervals with the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ).[27] Systolic and diastolic blood pressure were averaged from the last three measurements of each participant. Non-fasting blood samples were used to determine concentrations of insulin, glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides. Glucose, total cholesterol, HDL-cholesterol and triglycerides concentrations were measured using the c702 module on the Cobas 8000 analyzer. Insulin was measured with electrochemiluminescence immunoassay (ECLIA) on the E411 module (Roche, Almere, the Netherlands).[28]

Clustering of cardiovascular risk factors was defined as having three or more of the following components: android fat mass percentage 75th percentile or above; systolic or diastolic blood pressure 75th percentile or above; insulin concentration 75th percentile or above and HDL-cholesterol 25th percentile or below or triglycerides 75th percentile or above, based on previous literature.[17, 29] Clustering of cardiovascular risk factors was only calculated for those with complete data in all of these variables.

Brain imaging

This procedure has been described previously in detail.[20] Briefly, every child was invited to participate in a mock scanning session prior to the actual MRI scan to familiarize them with the procedure. If at any point the child was too anxious, he or she did not progress to the MRI

scan. Images were obtained using the same sequence on a 3-Tesla scanner (General Electric MR750w, Milwaukee, WI, USA). Following a three-plane localizer scan, a high resolution T1-weighted images were acquired with an inversion recovery fast-spoiled gradient recalled sequence (parameters: TR=8.77 ms, TE=3.4ms, TI=600ms, flip angle=10°, FOV=220 mm x 220 mm, acquisition matrix=220 x 220, slice thickness=1 mm, number of slices=230). At the scanner, T1 image quality was rated using a six-point Likert scale: unusable, poor, fair, good, very good, and excellent. If the initial T1-weighted scan was rated as unusable or poor, the T1 sequence was repeated.[20] Images were processed using FreeSurfer, version 6.0 (http://surfer.nmr.mgh.harvard.edu/), which had good test-retest reliability across scanners.[30] Additionally, the quality of FreeSurfer output was visually inspected, and images with insufficient quality were excluded. Independent raters were trained and systematically performed quality checks on the Freesurfer output of a random sample of 500 brain scans. The interobserver and intraobserver intraclass correlation coefficient were greater than 0.72.[20] Global metrics of volume, including total brain volume, total gray matter volume and total white matter volume and volumes of the specific cortical and subcortical gray structures, were extracted. DTI data were obtained using an echo-planar sequence with three b=0 scans and 35 diffusion-weighted images (b=1000 s/mm2) with the parameters: TR=12,500 ms, TE=72.8 ms, FOV=240 mm x 240 mm, acquisition matrix=120 x 120, slice thickness=2 mm, number of slices=65. DTI data were processed with the FMRIB Software Library (FSL) and the Camino diffusion MRI toolkit.[31, 32] The diffusion tensor was fit at each voxel, and common scalar metrics including global fractional anisotropy (FA) and mean diffusivity (MD) were computed. FA describes the degree of anisotropic diffusion and is highly sensitive to microstructural changes, and MD describes the average diffusion in all directions.[33, 34] White matter tracts, were determined using fully-automated probabilistic fiber tractography.[33, 35] Average FA and MD were calculated for each tract. Diffusion image quality was assessed by manual and automated inspection.

Covariates

Information on maternal age, educational level, pre-pregnancy BMI, prenatal psychological distress, prenatal smoking and prenatal alcohol consumption was obtained by questionnaires during pregnancy. Child sex, gestational age at birth, birth weight and date of birth, from which age at neuroimaging assessment was calculated, were available from medical records. Child ethnicity was based on parental countries of birth obtained through questionnaire at enrollment. Intracranial volume was obtained from MRI scans.

Statistical analysis

First, we performed a non-response analysis by comparing children with and without brain MRI measurements. Second, the associations of body fat and cardiovascular risk factors with brain outcomes were examined using linear regression analyses. We used a hierarchical approach to examine the associations of childhood body fat measures and cardiovascular

risk factors with brain outcomes. Global volumetric measures including total brain, total gray matter and total white matter volume, and overall white matter microstructure measures were examined in the primary analyses. If an association with a global metric was observed, post-hoc analyses of the specific cortical and subcortical structures were performed. We observed a quadratic relationship between BMI and brain global volumetric measures. Since the associations of childhood BMI with brain outcomes were not linear, we also used BMI categories (underweight, normal weight and overweight/obesity). For all analyses, we present a basic model (Model 1) including child´s sex and age at outcome measurements, and a confounder-adjusted model (Model 2), which additionally included maternal age and educational level and child birth weight and ethnicity. Potential confounders were represented in a directed acyclic graph (DAG) (**Figure S2** in Supplementary Materials) and were included if fulfilling the graphical criteria for confounding and changing the effect estimates >10% ($p<0.1$). Confounders were added to models using a stepwise approach. The correlation coefficients between confounders ranged from -0.37 to 0.34. Based on previous literature, we tested for statistical interactions with child sex in all models.[36] Overall, we found no statistically significant interactions (p-values>0.10) and thus no further stratified analyses were performed. Given the high correlation ($r >0.89$), models with global volumetric measures as outcomes were not adjusted for intracranial volume. Only models with subcortical regional volumes were adjusted for intracranial volume (Model 3).

As sensitivity analyses, we assessed whether the associations of childhood body fat measures, cardiovascular risk factors and brain outcomes were independent of maternal prenatal factors, by additionally adjusting our models for maternal pre-pregnancy BMI, psychological distress, smoking and alcohol consumption during pregnancy. To enable comparison of effect sizes of different body fat and cardiovascular exposure measures, we constructed standard deviation scores (SDS) ((observed value - mean)/SD), also known as z-scores. In addition, global white matter microstructure measures were also presented in SDS. Missing data in covariates (ranging from 0 to 2.5%) were imputed using Markov chain Monte Carlo approach with 10 imputations.[37] Pooled results were reported. We compared each p-value with a threshold defined as 0.05 divided by the effective number of independent tests estimated based on the correlation between the exposures (p-value threshold of .0077) to minimize false positive findings due to multiple testing.[38] We performed all statistical analyses using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS IBM, Chicago, IL, USA).

RESULTS

Subject characteristics

Table 1 shows that the sample consisted of 3,098 children (1,557 girls [50.3%]; mean age 9.8 years). Of all children, 68.6% had a European ethnic background, 6.5% had underweight, 17.3% had overweight or obesity and 13.7% had risk of clustering of cardiovascular risk factors. Non-response analyses showed that, as compared to children without brain MRI measurements, responders were more often appropriate for gestational age at birth, with a European ethnic background, with normal weight and had lower risk of clustering of cardiovascular risk factors (**Table S1** in Supplementary Materials).

Table 1. Characteristics of study population[1]

	Total group (N= 3,098)
Maternal characteristics	
Age, mean (SD), years	31.1 (4.9)
Education, N (%)	
Lower	186 (6.5)
Middle	1,156 (40.6)
Higher	1,506 (52.9)
Child characteristics	
Sex, N (%)	
Boys	1,541 (49.7)
Girls	1,557 (50.3)
Gestational age at birth, median (25^{th},75^{th} percentile), weeks	40.1 (39.1,41.0)
Birth weight[2], N (%)	
Small for gestational age	259 (8.4)
Appropriate for gestational age	2,488 (81.0)
Large for gestational age	325 (10.6)
Ethnicity, N(%)	
European	2,083 (68.6)
Non-European	953 (31.4)
Age at visit, mean (SD), years	9.8 (0.3)
BMI categories, N (%)	
Underweight	201 (6.5)
Normal weight	2,355 (76.2)
Overweight/Obesity	536 (17.3)
Fat mass index, median (25^{th},75^{th} percentile), kg/m^4	2.1 (1.7,2.8)
Android fat mass, mean (SD), %	4.3 (1.3)
Systolic blood pressure, mean (SD), mmHg	103.0 (7.9)
Diastolic blood pressure, mean (SD), mmHg	58.6 (6.5)

Table 1. Characteristics of study population[1] (*continued*)

	Total group (N= 3,098)
Insulin, median (25th,75th percentile), pmol/L	179.5 (107.3,291.0)
Glucose, mean (SD), mmol/L	5.3 (0.9)
Total-cholesterol, mean (SD),mmol/L	4.3 (0.6)
HDL-cholesterol, mean (SD), mmol/L	1.5 (0.3)
Triglycerides, median (25th,75th percentile), mmol/L	1.0 (0.7,1.3)
Clustering of cardiovascular risk factors, N (%)	
Yes	294 (13.7)
Global volumetric measures	
Total brain volume, mean (SD), cm^3	1,215 (110.3)
Total gray matter volume, mean (SD), cm^3	761.9 (64.3)
Cortical gray matter volume, mean (SD), cm^3	582.1 (53.5)
Subcortical gray matter volume, mean (SD), cm^3	60.5 (4.6)
Total white matter volume, mean (SD), cm^3	451.6 (50.7)
Intracranial volume, mean (SD), cm^3	1,512 (141.0)
Global white matter microstructure measures	
Global FA, mean (SD)	3.0×10^{-2} (1.8)
Global MD, mean (SD)	2.7×10^{-3} (0.2)

[1] Values are means (standard deviation), medians (25th,75th percentile) or numbers of subjects (valid %).
[2] Sex- and gestational age-adjusted birth weight SDS were created based on a North-European reference chart. Small and large size for gestational age at birth were defined as sex- and gestational age-adjusted birth weight below the 10th percentile and above the 90th percentile, respectively.

Body mass index, body fat distribution and brain outcomes

Table 2 shows an inverse U-shaped relation between childhood BMI and brain global volumetric measures, indicating that children with lower or higher BMI had smaller global brain volumes compared to children with a normal weight. As compared to children with a normal weight, those with underweight had a smaller total brain and white matter volumes (differences -18.10 (95% Confidence Interval (CI) -30.97,-5.22) cm^3, -10.64 (95% CI -16.82,-4.47) cm^3) respectively. Children with overweight and obesity had lower global white matter MD. Also, one SDS increase in android fat mass percentage was associated with lower white matter MD (difference -0.06 (95% CI -0.10,-0.02) SDS). Fat mass index was associated with smaller total brain and gray matter volumes (differences -3.89 (95% CI -7.34,-0.44) cm^3, -3.48 (95% CI -16.82,-4.47) cm^3, respectively) and with lower white matter FA. However, after multiple testing correction the associations of childhood overweight and obesity with lower white matter MD and higher fat mass index with lower total brain volume and white matter FA were attenuated into non-significant. Sensitivity analyses showed similar results after adjustment for maternal prenatal factors (**Table S2** in Supplementary Materials). Results from the basic models are given in the Supplemental **Table S3**.

Table 2. Associations of body mass index, body fat distribution and brain outcomes at 10 years[1]

	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
Linear model					
BMI (SDS)	4.82 (1.63,8.02)†**	1.86 (0.00,3.73)	2.93 (1.40,4.46)†**	-0.02 (-0.06,0.02)	-0.03 (-0.07,0.01)
Quadratic model					
BMI (SDS)	7.47 (4.03,10.92)†**	3.43 (1.43,5.44)†**	4.02 (2.36,5.67)†**	-0.01 (-0.05,0.03)	-0.02 (-0.07,0.02)
BMI^2 (SDS)	-4.42 (-6.60,-2.25)†**	-2.62 (-3.89,-1.35)†**	-1.81 (-2.86,-0.77)†**	-0.02 (-0.04,0.01)	-0.02 (-0.04,0.01)
BMI categories					
Underweight	-18.10 (-30.97,-5.22)†**	-7.44 (-14.93,0.05)	-10.64 (-16.82,-4.47)†**	0.01 (-0.15,0.17)	-0.01 (-0.17,0.15)
Normal weight	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight	-5.86 (-14.49,2.78)	-4.62 (-9.65,0.40)	-1.30 (-5.44,2.85)	-0.06 (-0.17,0.05)	-0.13 (-0.24,-0.03)*
Body fat distribution (SDS)					
Fat mass index	-3.89 (-7.34,-0.44)*	-3.48 (-5.49,-1.48)†**	-0.45 (-2.11,1.21)	-0.05 (-0.10,-0.01)*	-0.04 (-0.08,0.01)
Android fat percentage	-1.26 (-4.56,2.04)	-1.53 (-3.45,0.39)	0.23 (-1.36,1.81)	-0.02 (-0.07,0.02)	-0.06 (-0.10,-0.02)†**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for underweight and overweight/obese children compare to the reference group (normal weight) and for body mass index, fat mass index and android fat mass percentage in standard deviation scores. Models are adjusted for maternal age and educational level and child sex, age, ethnicity and birth weight. *$p < 0.05$. ** $p < 0.01$. † These results survived multiple testing correction.

The post-hoc analyses showed that children with overweight and obesity had smaller frontal lobe volumes. Moreover, one SDS increase in fat mass index was associated with smaller frontal and temporal lobes volumes (all p-values < 0.05) (**Table S4** in Supplementary Materials). After additionally adjusting for intracranial volume, children with underweight had smaller amygdala, hippocampal and globus pallidus volumes. In contrast, children with overweight and obesity had larger amygdala and hippocampal volumes. Also, one SDS increase in fat mass index was associated with larger thalamus, amygdala, hippocampal and putamen volumes (all p values < 0.05) (**Table S5** in Supplementary Materials).

Cardiovascular risk factors and brain outcomes

Table 3 shows that of all cardiovascular risk factors, only childhood diastolic blood pressure was associated with smaller total gray matter volume and triglycerides concentrations was associated with larger total gray matter volume ($p < 0.05$). However, after multiple testing correction, none of these associations remained significant. Similar results were observed in the sensitivity analyses (**Table S6** in Supplementary Materials). Results from the basic models are given in the Supplemental **Table S7**.

DISCUSSION

In this population-based study, we showed that body fat measures were associated with brain structure in school-age children. Specifically, our findings suggest that, as compared to children with a normal weight, children with underweight have a smaller total brain and white matter volumes. Also, one SDS increase in fat mass index was associated with smaller gray matter volumes, and one SDS increase in android fat mass percentage was related to lower mean diffusivity of the white matter.

Interpretation of main findings

As a result of the childhood obesity epidemic, cardiovascular risk factors, such as high blood pressure, insulin resistance and dyslipidemia are increasing among children and adolescents.[1, 2] Negative brain consequences of poorer cardiovascular profile have been reported later in life.[4-7] We hypothesized that an adverse body fat distribution or cardiovascular risk factors might be related with brain development already in childhood.

A recent cross-sectional study among 2,700 children aged 9–11 years from the ABCD dataset in the United Sates suggested an association of higher BMI with reduced mean cortical thickness and lower executive function.[12] Higher BMI has also been linked with brain structural changes, specifically reductions in gray matter volume and in white matter microstructural integrity among children and adolescents.[8-11] In the current study, we observed that not only BMI but also body fat distribution measures such as fat mass index and android fat mass

Table 3. Associations of cardiovascular risk factors and brain outcomes at 10 years[1]

	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total Brain volume	Total Gray Matter	Total White Matter	Fractional Anisotropy	Mean Diffusivity
Cardiovascular risk factors (SDS)					
Systolic blood pressure	-0.40 (-3.64,2.84)	-0.05 (-1.93,1.83)	-0.37 (-1.92,1.19)	-0.03 (-0.07,0.01)	0.01 (-0.03,0.05)
Diastolic blood pressure	-3.21 (-6.43,0.01)	-1.91 (-3.78,-0.03)*	-1.32 (-2.87,-0.23)	-0.03 (-0.07,0.01)	0.01 (-0.03,0.05)
Insulin	-1.98 (-5.70,1.74)	-1.35 (-3.51,0.82)	-0.63 (-2.43,1.16)	-0.04 (-0.09,0.00)	-0.01 (-0.06,0.03)
Glucose	1.08 (-2.60,4.76)	0.63 (-1.51,2.76)	0.46 (-1.31,2.24)	-0.03 (-0.07,0.02)	0.01 (-0.03,0.06)
Total Cholesterol	1.60 (-2.09,5.28)	0.75 (-1.39,2.89)	0.84 (-0.94,2.61)	-0.01 (-0.06,0.03)	0.04 (-0.01,0.08)
HDL Cholesterol	-2.01 (-5.72,1.70)	-1.43 (-3.59,0.73)	-0.57 (-2.36,1.22)	0.04 (-0.00,0.09)	0.00 (-0.04,0.05)
Triglycerides	3.24 (-0.44,6.93)	2.53 (0.39,4.67)*	0.71 (-1.07,2.49)	-0.03 (-0.08,0.01)	0.01 (-0.04,0.05)
Clustering of cardiovascular risk					
No	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Yes	2.07 (-9.07,13.21)	0.89 (-5.57,7.34)	1.15 (-4.24,6.53)	-0.04 (-0.18,0.10)	-0.06 (-0.20,0.08)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for cardiovascular risk factors in standard deviation scores and for clustering of cardiovascular risk, compared to the reference group. Models are adjusted for maternal age and educational level and child sex, age, ethnicity and birth weight. *$p < 0.05$. ** $p < 0.01$.

percentage were associated with brain structure in school-age children. We observed that one SDS increase in fat mass index was associated with smaller gray matter volumes, specifically smaller frontal and temporal lobes volumes and one SDS increase in android fat mass percentage was related to lower mean diffusivity of the white matter. Our results were in line with previous studies that showed an association of adolescent obesity with changes in cortical grey matter volume and connectivity, commonly in prefrontal regions known to be associated with executive function.[10, 11] In addition, we reported that childhood underweight was also related to smaller total brain volume, particularly white matter volume. Our findings are in line with a recent study from the same cohort showing inverse U-shaped associations of childhood BMI with less cortical gyrification, potentially suggesting that both lower and higher BMI might be associated with different brain development trajectories in childhood.[16]

Elevated blood pressure, insulin resistance and dyslipidemia in early adulthood are known to be associated with brain health in elderly.[4-6] However, little is known about childhood cardiovascular risk factors and brain morphology. Previous results from the Generation R Study showed an association between higher diastolic blood pressure and lower cognitive function in children.[15] In the present study, we observed that higher diastolic blood pressure tended to be associated with smaller total gray matter volume. However, the association disappeared after multiple testing correction. A previous study among 111 nondiabetic adolescents (49 with and 62 without metabolic syndrome) suggested that insulin resistance was the most significant predictor of brain volumetric changes, specifically smaller hippocampal volumes.[14] Another retrospective study observed no significant differences between 108 adolescents with type 1 diabetes and 51 healthy control subjects in regional grey and white matter volumes.[39] Also, a cross-sectional study among 1,297 Taiwanese adolescents did not observe associations between total cholesterol level and cognitive abilities.[13] In the current study, none of the cardiovascular risk factors were associated with the brain outcomes after adjustment for multiple testing. A possible explanation for our findings is that subtle differences in cardiovascular risk factors may not be enough to trigger manifestations of poorer brain health.

It has been proposed that increased inflammation, hormonal changes and oxidative stress contribute to endothelial dysfunction and vascular reactivity affecting brain structural integrity and function.[40, 41] In children, obesity has been linked to low-grade inflammation, increased C-reactive protein and alterations in endocrine hormones.[42, 43] Likewise, a study among anorexic adolescents showed that underweight was related to higher levels of interleukin-6, apolipoprotein-B and global endocrine dysregulation.[44] Therefore, it could be that in the current study the observed negative association of underweight and overweight in relation to brain volumes in childhood might be explained by such inflammatory and endocrine mechanisms.

Altogether, our results suggest that body weight and body fat distribution, but not other cardiovascular risk factors, may affect brain morphology and white matter microstructure

in children. Our findings were independent of several maternal and child factors. However, the observed effect estimates may be small on an individual level, but can be important on a population-based level. Studying body weight and body fat distribution during childhood presents a unique opportunity to evaluate whether brain structure may be already affected by metabolic dysregulation before the development of clinically manifest cardiovascular disease. Due to the observational design of this study, we cannot infer causality. Prospective studies are needed to determine how patterns of body weight and body fat distribution over time may relate morphological differences to cognitive function of specific brain regions.

Strengths and limitations

The major strength of this study was the assessment of detailed measurements of a variety of cardiovascular risk factors in combination with brain imaging in a large pediatric population-based study. Also, we were able to adjust for several confounding factors, including both maternal and child factors. However, several limitations should be mentioned. First, because of the observational and cross-sectional study design, we could not draw strong conclusions about causality and temporality of the observed associations. Future studies with longitudinal follow-up data may inform us about the temporality of the associations and intervention studies, preferably randomized controlled trials, will inform us about the causal nature of the observed associations. Second, we used non-fasting blood samples instead of fasting blood samples, which might reduce the accuracy of the cardiovascular risk factor measurements. Although non-fasting samples are frequently used in population-based studies, they may lead to less precision in the exposure assessments. Third, although childhood brain structure has been significantly linked to cognitive, emotional, and sensorimotor functions in the general population, we did not address the functional implications of the observed morphological differences.[45] While the current study was specifically focused on examining brain structure, future studies should investigate the associations of cardiovascular indicators with repeated assessments of brain morphology, as well as multi-modal neuroimaging in combination with behavioral and cognitive functioning in large population-based cohorts. Fourth, we utilized the default templates and atlases for neuroimaging analysis. Reports suggest that using age study-specific templates has some advantages, such as minimizing bias. However there is still no consensus where the bias in brain imaging arises from (the nature of the template, or the performance of a software package with a given template/atlas).[46, 47] Fifth, our population was relatively high educated and more often of Dutch origin, suggesting a possible selection bias due to refraining from participation in the neuroimaging visit. This makes generalization of the results challenging. Finally, we collected information on many potential confounding variables, but residual confounding due to unmeasured lifestyle variables might still be an issue.

CONCLUSION

Results from this cross-sectional population-based study suggest that body weight and body fat distribution relate to children's brain morphology and white matter microstructure at the age of 10 years. Further research is needed to assess causality, temporality and potential long-term consequences on brain functioning.

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SUPPLEMENTARY MATERIALS

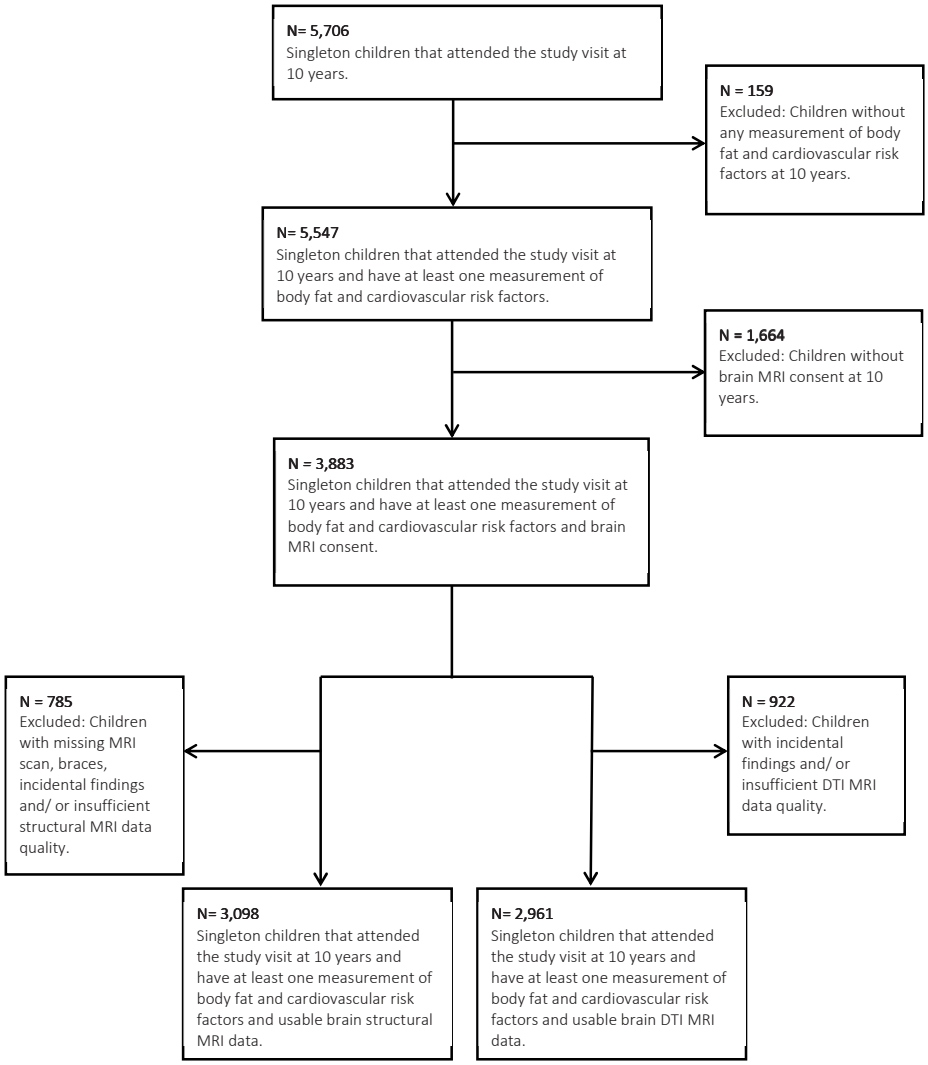


Figure S1. Flowchart of study population

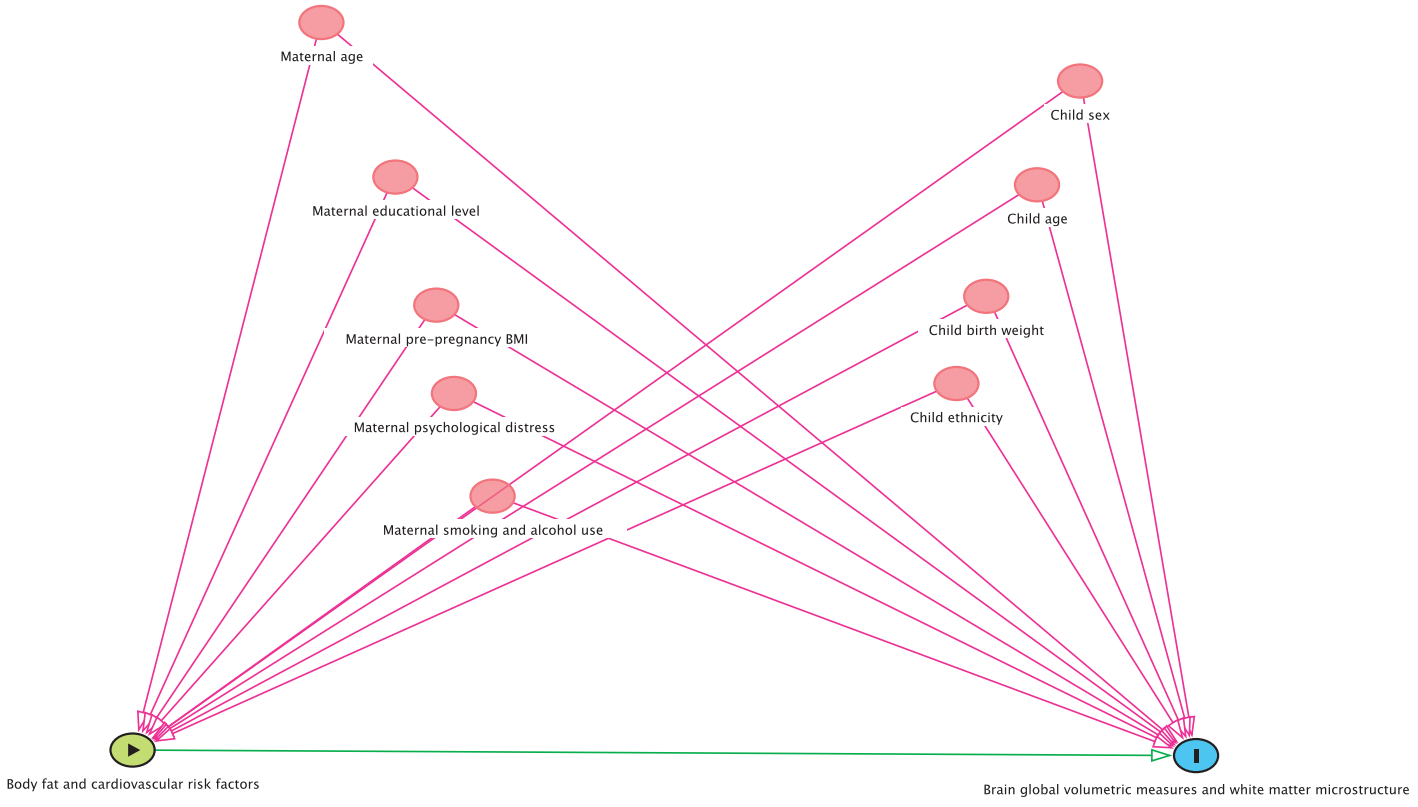


Figure S2. Simplified Directed Acyclic Graph showing the hypothesized relationship between childhood body fat and cardiovascular risk factors and brain outcomes and the covariates

Table S1. Comparison of characteristics between responders and non-responders[1]

	Responders (N= 3,098)	Non-Responders (N= 2,449)	P-value[2]
Maternal characteristics			
Age, mean (SD), years	31.1 (4.9)	30.8 (5.1)	0.08
Educational level, N (%)			< 0.01
Lower	186 (6.5)	197 (8.7)	
Middle	1,156 (40.6)	996 (44.0)	
Higher	1,506 (52.9)	1,069 (47.3)	
Child characteristics			
Sex, N (%)			0.98
Boys	1,541 (49.7)	1,219 (49.8)	
Girls	1,557 (50.3)	1,230 (50.2)	
Gestational age at birth, median (25^{th},75^{th} percentile), weeks	40.1 (39.1,41.0)	40.1 (35.4,42.3)	0.07
Birth weight[3], N (%)			0.19
Small for gestational age	259 (8.4)	239 (9.8)	
Appropriate for gestational age	2,488 (81.0)	1,933 (79.5)	
Large for gestational age	325 (10.6)	258 (10.6)	
Ethnicity, N (%)			0.13
European	2,083 (68.6)	1,588 (66.7)	
Non-European	953 (31.4)	794 (33.3)	
Age at visit, mean (SD), years	9.8 (0.3)	9.8 (0.3)	< 0.01
BMI categories, N (%)			0.06
Underweight	201 (6.5)	177 (7.3)	
Normal weight	2,355 (76.2)	1,790 (73.3)	
Overweight/Obesity	536 (17.3)	474 (19.4)	
Fat mass index, median (25^{th},75^{th} percentile), kg/m^4	2.1 (1.7,2.8)	2.2 (1.7,3.0)	< 0.01
Android fat mass, mean (SD), %	4.3 (1.3)	4.5 (1.4)	< 0.01
Systolic blood pressure, mean (SD), mmHg	103.0 (7.9)	103.3 (8.0)	0.15
Diastolic blood pressure, mean (SD), mmHg	58.6 (6.5)	58.6 (6.4)	0.83
Insulin, median (25^{th},75^{th} percentile), pmol/L	179.5 (107.3,291.0)	168.8 (99.3,269.4)	< 0.01
Glucose, mean (SD), mmol/L	5.3 (0.9)	5.1 (0.9)	< 0.01
Total-cholesterol, mean (SD),mmol/L	4.3 (0.6)	4.3 (0.7)	0.45
HDL-cholesterol, mean (SD), mmol/L	1.5 (0.3)	1.5 (0.4)	0.17
Triglycerides, median (25^{th},75^{th} percentile), mmol/L	1.0 (0.7,1.3)	1.0 (0.7,1.4)	< 0.01
Risk of cardiovascular risk factors, N (%)			0.22
Yes	294 (13.7)	232 (15.1)	

[1] Values are means (standard deviation), medians (25^{th},75^{th} percentile) or numbers of subjects (valid %).

[2] P-values for differences in subject characteristics between groups were calculated performing independent sample t-tests for normally distributed continuous variables, Mann-Whitney tests for not normally distributed continuous variables and chi-square tests for categorical variables.

[3]Sex- and gestational age-adjusted birth weight SDS were created based on a North-European reference chart. Small and large size for gestational age at birth were defined as sex- and gestational age-adjusted birth weight below the 10^{th} percentile and above the 90^{th} percentile, respectively.

Table S2. Associations of body mass index, body fat distribution and brain outcomes at 10 years, sensitivity analyses1

	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
Linear model					
BMI (SDS)	6.05 (2.58,9.51)**	2.70 (0.69,4.72)**	3.31 (1.66,4.96)**	-0.02 (-0.06,0.02)	-0.03 (-0.08,0.01)
Quadratic model					
BMI (SDS)	8.35 (4.71,11.98)**	4.05 (1.93,6.17) **	4.27 (2.53,6.01)**	-0.01 (-0.05,0.04)	-0.02 (-0.07,0.02)
BMI^2 (SDS)	-4.13 (-6.33,-1.94)**	-2.42 (-3.70,-1.14)**	-1.72 (-2.78,-0.67)**	-0.02 (-0.04,0.01)	-0.02 (-0.04,0.01)
BMI categories					
Underweight	-18.63 (-31.58,-5.69)**	-7.91 (-15.44,-0.37)*	-10.72 (-16.92,-4.51)**	0.01 (-0.15,0.17)	-0.01 (-0.17,0.16)
Normal weight	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight	-4.21 (-13.18,4.76)	-3.37 (-8.58,1.85)	-0.90 (-5.19,3.39)	-0.05 (-0.16,0.06)	-0.13 (-0.24,-0.02)*
Body fat distribution (SDS)					
Fat mass index	-3.28 (-7.02,0.45)	-3.01 (-5.17,-0.84)**	-0.32 (-2.10,1.46)	-0.05 (-0.10,-0.01)*	-0.04 (-0.08,0.01)
Android fat percentage	-0.48 (-3.97,3.00)	-0.95 (-2.98,1.01)	0.42 (-1.25,2.09)	-0.02 (-0.06,0.02)	-0.07 (-0.11,-0.02)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for underweight and overweight/obese children compare to the reference group (normal weight) and for body mass index, fat mass index and android fat mass percentage in standard deviation scores. Models are adjusted for maternal age, educational level, pre-pregnancy BMI, prenatal psychological distress, prenatal smoking and prenatal alcohol consumption and child sex, age, ethnicity and birth weight. *$p < 0.05$. ** $p < 0.01$.

Table S3. Associations of body mass index, body fat distribution and brain outcomes at 10 years, basic models1

	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total brain volume	Total gray matter	Total white matter	Fractional anisotropy	Mean diffusivity
Linear model					
BMI (SDS)	0.95 (-2.33,4.23)	-0.87 (-2.80,1.05)	1.80 (0.20,3.33)*	-0.04 (-0.08,0.01)	-0.02 (-0.06,0.02)
Quadratic model					
BMI (SDS)	6.01 (2.49,9.64)**	2.23 (0.14,4.33)*	3.81 (2.13,5.49)**	-0.02 (-0.07,0.02)	-0.01 (-0.05,0.03)
BMI^2 (SDS)	-7.99 (-10.29,-5.69)**	-4.85 (-6.20,-3.50)**	-3.15 (-4.22,-2.07)**	-0.03 (-0.06,-0.00)*	-0.02 (-0.04,0.01)
BMI categories					
Underweight	-27.85 (-41.55,-14.15)**	-12.97 (-21.01,-4.94)**	-14.86 (-21.28,-8.45)**	-0.00 (-0.16,0.16)	-0.04 (-0.20,0.12)
Normal weight	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight	-19.60 (-28.55,-10.66)**	-13.90 (-19.15,-8.65)**	-5.76 (-9.95,-1.57)**	-0.13 (-0.24,-0.03)*	-0.11 (-0.22,-0.01)*
Body fat distribution (SDS)					
Fat mass index	-12.2 (-15.6,-8.70)**	-8.86 (-10.9,-6.84)**	-3.33 (-4.96,-1.70)**	-0.09 (-0.13,-0.05)**	-0.03 (-0.07,0.01)
Android fat percentage	-7.64 (-11.07,-4.22)**	-5.69 (-7.70,-3.69)**	-1.99 (-3.60,-0.38)*	-0.05 (-0.09,-0.01)**	-0.06 (-0.10,-0.02)**

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for underweight and overweight/obese children compare to the reference group (normal weight) and for body mass index, fat mass index and android fat mass percentage in standard deviation scores. Models are adjusted for child sex and age. *$p < 0.05$. ** $p < 0.01$.

Table S4. Associations of body mass index, body fat distribution and brain cortical regional volumetric measures at 10 years[1]

		Cortical Regional Volumetric Measures (cm^3)				
		Cortical Gray volume	Frontal lobe volume	Parietal lobe volume	Temporal lobe volume	Occipital lobe volume
BMI categories						
Underweight	Model 1	-10.0 (-16.8,-3.22)**	-3.77 (-6.52,-1.01)**	-2.46 (-4.47,-0.44)*	-2.37 (-4.00,-0.75)**	-0.30 (-1.24,0.64)
	Model 2	-5.55 (-12.0,0.89)	-2.20 (-4.85,0.45)	-1.04 (-2.94,0.86)	-1.51 (-3.08,0.06)	-0.01 (-0.92,0.91)
Normal weight	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight	Model 1	-10.67 (-15.1,-6.23)**	-4.69 (-6.49,-2.90)**	-3.05 (-4.36,-1.73)**	-1.71 (-2.77,-0.65)**	-0.68 (-1.29,-0.06)*
	Model 2	-5.65 (-9.91,-1.40)**	-2.95 (-4.71,-1.20)**	-1.60 (-2.86,-0.35)	-0.74 (-1.78,0.30)	-0.07 (-0.67,0.54)
Body fat distribution (SDS)						
Fat mass index	Model 1	-6.60 (-8.32,-4.88)**	-2.82 (-3.51,-2.13)**	-1.74 (-2.25,-1.23)**	-1.26 (-1.67,-0.86)**	-0.44 (-0.68,-0.20)**
	Model 2	-3.29 (-4.97,-1.60)**	-1.69 (-2.39,-1.00)**	-0.76 (-1.26,-0.26)	-0.63 (-1.05,-0.22)**	-0.04 (-0.29,0.19)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain cortical regional volumetric measures for underweight and overweight/obese children compare to the reference group (normal weight) and for fat mass index in standard deviation scores. *$p < 0.05$. ** $p < 0.01$.

Model 1: adjusted for child sex and age.

Model 2: adjusted for maternal age and educational level and child sex, age, ethnicity and birth weight.

Table S5. Associations of body mass index, body fat distribution and brain subcortical regional volumetric measures at 10 years[1]

		Subcortical Regional Volumetric Measures (cm^3)							
		Subcortical Gray volume	Thalamus volume	Amygdala volume	Hippocampus volume	Globus Pallidus volume	Putamen volume	Caudate nucleus volume	Nucleus accumbens volume
BMI categories									
Underweight	Model 1	-1.20 (-1.80,-0.60)**	-0.28 (-0.46,-0.10)**	-0.11 (-0.17,-0.06)**	-0.17 (-0.27,-0.07)**	-0.10 (-0.16,-0.05)**	-0.24 (-0.39,-0.09)**	-0.10 (-0.24,0.04)	-0.03 (-0.06,-0.00)*
	Model 2	-0.85 (-1.43,-0.26)**	-0.18 (-0.35,-0.00)*	-0.10 (-0.15,-0.05)**	-0.14 (-0.24,-0.04)**	-0.07 (-0.13,-0.02)*	-0.19 (-0.33,-0.04)*	-0.04 (-0.17,0.10)	-0.02 (-0.04,0.01)
	Model 3	-0.45 (-0.85,-0.06)*	-0.07 (-0.20,0.06)	-0.08 (-0.12,-0.04)**	-0.09 (-0.17,-0.01)*	-0.05 (-0.10,0.00)*	-0.13 (-0.27,0.01)	0.03 (-0.09,0.14)	-0.01 (-0.03,0.02)
Normal weight		*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight	Model 1	-0.59 (0.98,-0.20)**	-0.21 (-0.33,-0.10)**	0.00 (-0.03,0.04)	-0.05 (-0.11,0.02)	-0.03 (-0.06,0.01)	-0.04 (-0.14,0.06)	-0.17 (-0.26,-0.08)**	-0.02 (-0.04,-0.01)*
	Model 2	-0.31 (-0.69,0.08)	-0.11 (-0.23,0.00)	0.03 (-0.01,0.06)	-0.01 (-0.07,0.06)	-0.02 (-0.06,0.01)	-0.02 (-0.12,0.08)	-0.12 (-0.21,-0.03)**	-0.01 (-0.03,0.00)
	Model 3	0.24 (-0.02,0.50)	0.04 (-0.05,0.12)	0.06 (0.03,0.09)**	0.06 (0.01,0.12)*	0.01 (-0.02,0.04)	0.06 (-0.03,0.15)	-0.03 (-0.10,0.05)	0.00 (-0.02,0.02)
Body fat distribution (SDS)									
Fat mass index	Model 1	-0.45 (-0.61,-0.30)**	-0.15 (-0.19,-0.10)**	-0.01 (-0.02,0.01)	-0.04 (-0.07,-0.02)*	-0.02 (-0.04,-0.01)**	-0.03 (-0.07,0.01)	-0.12 (-0.15,-0.08)**	-0.02 (-0.02,-0.01)**
	Model 2	-0.26 (-0.42,-0.11)**	-0.08 (-0.13,-0.04)**	0.01 (-0.00,0.02)	-0.01 (-0.04,0.01)	-0.02 (-0.03,-0.00)*	-0.02 (-0.05,0.03)	-0.08 (-0.12,-0.05)**	-0.01 (-0.02,-0.00)*
	Model 3	0.16 (0.06,0.27)**	0.03 (0.00,0.07)*	0.04 (0.02,0.05)**	0.04 (0.02,0.06)**	0.01 (-0.01,0.02)	0.05 (0.01,0.08)*	-0.01 (-0.04,0.02)	0.00 (-0.00,0.01)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm3 of childhood brain subcortical regional volumetric measures for underweight and overweight/obese children compare to the reference group (normal weight) and for fat mass index in standard deviation scores. *p < 0.05. ** p < 0.01.

Model 1: adjusted for child sex and age.

Model 2: adjusted for maternal age and educational level and child sex, age, ethnicity and birth weight.

Model 3: Model 2 additionally adjusted for intracranial volume.

Table S6. Associations of cardiovascular risk factors and brain outcomes at 10 years, sensitivity analyses[1]

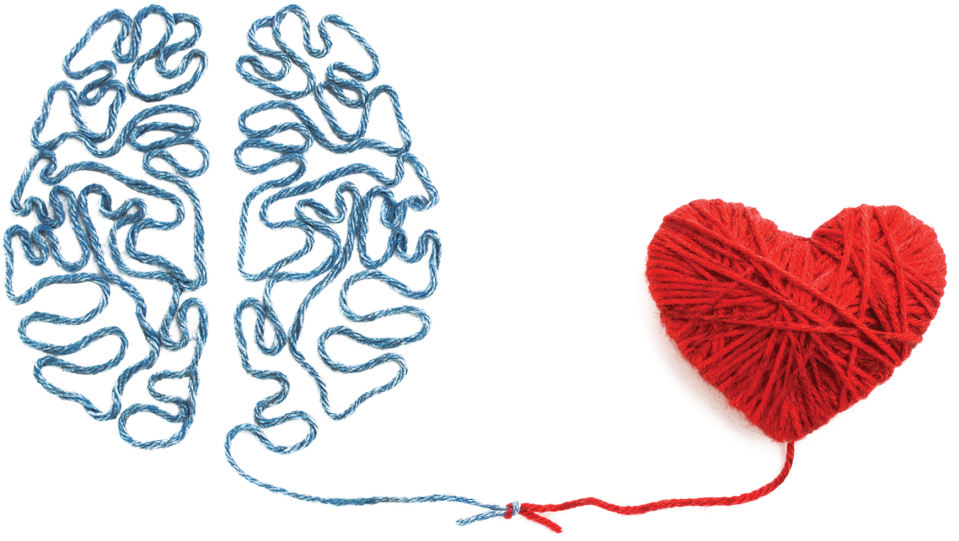
	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total Brain volume	Total Gray Matter	Total White Matter	Fractional Anisotropy	Mean Diffusivity
Cardiovascular risk factors (SDS)					
Systolic blood pressure	-0.16 (-3.45,3.13)	0.18 (-1.73,2.09)	-0.37 (-1.95,1.22)	-0.03 (-0.07,0.01)	0.01 (-0.03,0.05)
Diastolic blood pressure	-2.98 (-6.22,0.26)	-1.72 (-3.60,0.17)	-1.28 (-2.83,0.28)	-0.03 (-0.07,0.01)	0.01 (-0.03,0.05)
Insulin	-2.02 (-5.76,1.72)	-1.35 (-3.52,0.83)	-0.67 (-2.48,1.13)	-0.04 (-0.09,0.00)	-0.01 (-0.06,0.03)
Glucose	1.06 (-2.62,4.75)	0.61 (-1.53,2.75)	0.47 (-1.31,2.24)	-0.03 (-0.07,0.02)	0.01 (-0.03,0.06)
Total Cholesterol	1.62 (-2.06,5.30)	0.77 (-1.37,2.90)	0.85 (-0.92,2.63)	-0.01 (-0.06,0.03)	0.04 (-0.01,0.08)
HDL Cholesterol	-2.30 (-6.05,1.45)	-1.67 (-3.84,0.51)	-0.63 (-2.44,1.19)	0.04 (-0.00,0.09)	0.00 (-0.05,0.05)
Triglycerides	3.57 (-0.15,7.30)	2.78 (0.62,4.94)*	0.79 (-1.01,2.59)	-0.03 (-0.08,0.02)	0.01 (-0.04,0.05)
Clustering of cardiovascular risk					
No	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Yes	2.57 (-8.81,13.95)	1.39 (-5.19,7.97)	1.15 (-4.35,6.66)	-0.04 (-0.18,0.10)	-0.06 (-0.20,0.08)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for cardiovascular risk factors in standard deviation scores and for clustering of cardiovascular risk, compared to the reference group. Models are adjusted for maternal age, educational level, pre-pregnancy BMI, prenatal psychological distress, prenatal smoking and prenatal alcohol consumption and child sex, age, ethnicity and birth weight. *$p < 0.05$. ** $p < 0.01$.

Table S7. Associations of cardiovascular risk factors and brain outcomes at 10 years, basic models[1]

	Global volumetric measures (cm^3)			Global white matter microstructure measures (SDS)	
	Total Brain volume	Total Gray Matter	Total White Matter	Fractional Anisotropy	Mean Diffusivity
Cardiovascular risk factors (SDS)					
Systolic blood pressure	-3.37 (-6.81,0.07)	-2.03 (-4.05,-0.13)*	-1.36 (-2.97,0.26)	-0.05 (-0.08,-0.01)*	0.01 (-0.03,0.05)
Diastolic blood pressure	-7.00 (-10.42,-3.58)**	-4.36 (-6.36,-2.35)**	-2.65 (-4.26,-1.05)**	-0.05 (-0.09,-0.01)*	0.01 (-0.03,0.05)
Insulin	-5.98 (-9.95,-2.02)**	-3.97 (-6.30,-1.64)**	-2.02 (-3.88,-0.16)*	-0.06 (-0.11,-0.02)**	-0.01 (-0.06,0.03)
Glucose	0.87 (-3.08,4.83)	0.52 (-1.81,2.84)	0.36 (-1.49,2.22)	-0.03 (-0.07,0.02)	0.01 (-0.03,0.06)
Total Cholesterol	0.16 (-3.79,4.10)	-0.14 (-2.46,2.18)	0.29 (-1.56,2.14)	-0.02 (-0.06,0.03)	0.04 (-0.01,0.08)
HDL Cholesterol	-0.08 (-4.06,3.90)	-0.14 (-2.48,2.20)	0.07 (-1.80,1.94)	0.05 (0.01,0.10)*	0.00 (-0.04,0.05)
Triglycerides	1.03 (-2.92,4.98)	1.16 (-1.16,3.48)	-0.14 (-1.99,1.71)	-0.04 (-0.09,0.00)	0.00 (-0.04,0.05)
Clustering of cardiovascular risk					
No	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Yes	-11.78 (-23.57,0.02)	-8.13 (-15.04,-1.21)*	-3.68 (-9.23,1.87)	-0.10 (-0.24,0.03)	-0.06 (-0.20,0.07)

[1]Values are linear regression coefficients (95% confidence intervals) and reflect the change in cm^3 of childhood brain global volumetric and in standard deviation scores of global white matter microstructure measures for cardiovascular risk factors in standard deviation scores and for clustering of cardiovascular risk, compared to the reference group. Models are adjusted for child sex and age. *p < 0.05. ** p < 0.01.



Chapter 4

General Discussion

BACKGROUND

Cardiovascular disease is a major public health problem and is the leading cause of mortality and morbidity in the general adult population worldwide.[1] Next to cardiovascular disease, neurodegenerative disorders are also a leading cause of death and disability among the elderly.[2] Cardiovascular and brain health are related and share important risk factors such as obesity, hypertension, diabetes and dyslipidemia.[3, 4] Due to the largest clinical impact at older ages, research into risk factors for cardiovascular and brain health has mostly been focused on adults. However, in the last decades, an accumulating body of evidence suggested that cardiovascular and brain health in younger ages also has major public health implications.[5-9]

It has been proposed that adverse cardiovascular and neurodevelopmental outcomes in childhood and adulthood may originate in early life.[10, 11] According to the Developmental Origins of Health and Disease (DOHaD) hypothesis, adverse early-life exposures lead to developmental adaptation mechanisms that may have short and long term consequences for growth, body composition and cardiovascular and brain health in later life.[12-14] Assessing the influence of early-life determinants on detailed cardiovascular and brain outcomes measures, already in childhood, is important to evaluate whether adverse early-life exposures may trigger manifestations of poorer health before the development of clinically manifest diseases.

Therefore, the general aim of this thesis was to identify early-life factors, in particular related to psychological distress, growth, lifestyle or chemical exposures, that play an important role in the development of suboptimal cardiovascular and brain health outcomes in childhood. This chapter provides a general discussion of the main findings presented in this thesis, discusses general methodological issues and suggests direction for future research and potential implications for clinical practice and policy.

INTERPRETATION OF MAIN FINDINGS

Childhood cardiovascular health

Maternal psychological distress

Psychological distress is common during pregnancy, affecting 10%–20% of pregnant women, and is mostly defined as perceived stress, depressive symptoms, anxiety, or experiencing an adverse life event.[15-17] During pregnancy, psychological distress is suggested to increase maternal glucocorticoid levels which may affect the hypothalamic-pituitary-adrenal (HPA) axis activity in the fetus leading to long-term consequences on growth and cardiovascular health of the offspring.[18, 19] Previous studies suggested a link between fetal exposure to increased glucocorticoids and an increased risk of obesity and cardiovascular disease in adults.[20-22] Although some studies have also observed an increased risk of excess adiposity,

altered blood pressure and insulin resistance in children and adolescents exposed to prenatal psychological distress, results are not consistent.[23-28] Therefore, in **Chapter 2.1** and **Chapter 2.2** of this thesis, we assessed whether maternal psychological distress during pregnancy is associated with childhood body fat measures and cardiovascular risk factors at age 10 years. We observed that maternal psychological distress and anxiety during pregnancy were associated with higher general fat measures and an increased risk of overweight and obesity in the offspring. These findings are consistent with a previous study among over 65,000 mother-child pairs, which showed an association between maternal stress during pregnancy, defined by maternal bereavement, and an increased risk of overweight in the offspring at 10 to 13 years of age.[28] However, the same study found no significant association between prenatal maternal stress and the risk of overweight in children younger than 10 years.[28] Likewise, previous results from our own research group reported no association between maternal prenatal stress and offspring body mass index (BMI) in children aged 3 months to 3 years.[25] Thus, maternal psychological distress during pregnancy seems not to influence adiposity development in early childhood, but the effects seem to become more apparent at older offspring ages. We also observed that maternal anxiety, but not overall psychological distress and depression, was associated with higher childhood subcutaneous fat index, visceral fat index, and liver fat fraction at 10 years. Even though previous studies suggest that anxiety and depression share a common biological and genetic background, they are not identical emotional states.[29] The consistent associations observed in our study for anxiety with general and organ fat measures and the absence of associations for depression suggest that the mechanisms might be dependent on the specific psychiatric symptoms experienced or might be more pronounced for anxiety symptoms.

In this thesis, we observed that maternal psychological distress was not associated with offspring blood pressure, cholesterol, insulin, glucose and C-reactive protein concentrations in childhood. However, maternal psychological distress, depression and anxiety were associated with higher heart rate among boys and maternal anxiety was also associated with higher triglycerides among girls. Different mechanisms have been proposed to explain the sex-specific responses to maternal stress.[30, 31] For example, female, but not male placenta, adjusts their glucocorticoid metabolic activity in the presence of high maternal glucocorticoid levels.[32, 33] Also, the higher growth rates and diminished flexibility of male fetuses may increase their vulnerability during disturbed pregnancies.[32]

Several mechanisms underlying the observed associations of maternal psychological distress during pregnancy with offspring body fat and cardiovascular outcomes have been proposed.[34, 35] One of the most described mechanisms includes fetal hypothalamic-pituitary-adrenal (HPA) axis dysregulation in response to increased maternal stress hormones such as cortisol.[36, 37] Ideally, in a healthy pregnancy, maternal glucocorticoid levels are markedly higher than those in the fetal circulation due to the placental inactivation of active cortisol into its inactive form, cortisone.[36] However, several factors, such as prenatal stress, may alter

this mechanism allowing cortisol to cross the placenta and elevate fetal glucocorticoid levels.[35, 38] Cortisol can disturb the development of the fetal HPA axis by changing the HPA axis's negative feedback mechanism and the glucocorticoid receptor expression potentially affecting the postnatal activity of the fetal HPA axis.[39] It has been proposed that individuals exposed to stress in early life may have reduced expression of glucocorticoid receptor in the hippocampus which could lead to a reduction of the HPA axis negative feedback and an overactive HPA axis.[40] Another potential mechanism is the programming of the fetal autonomic nervous system, specifically changes in the balance of sympathetic and parasympathetic nervous system, in response to stress.[34] Within seconds after a stressor, catecholamines are produced by the sympathetic nervous system, providing a pathway that allows rapid regulation of vital functions, such as increasing heart and respiratory rates as well as catabolism.[41] Catecholamines are also linked to the HPA axis, which responds within minutes to hours, enhancing the catecholamines activity.[42] Thus, the HPA axis and autonomic nervous system are highly interconnected and constitute the main mechanisms of the normal adaptive response to acute stress.[43] However, prolongation of this response may lead to deleterious effects on body metabolism, potentially increasing the risk of obesity and adverse cardiovascular outcomes.[34, 44]

Overall, the findings of this thesis suggest that maternal psychological distress during pregnancy may influence body fat, and to a less extent and in a sex-specific manner, cardiovascular profile, during childhood.

Growth patterns

Infancy and early childhood seem also to be critical periods for the development of adverse cardiovascular outcomes in childhood and cardiovascular disease in adulthood.[45-48] Previous results from our own research group suggested that higher infant peak weight velocity and BMI at adiposity peak are associated with an increased risk of childhood overweight, as well as with cardiovascular outcomes, such as higher blood pressure and lower left ventricular mass, already at school-age.[49, 50] Markers of arterial health, such as carotid intima-media thickness and distensibility, have also been used to assess cardiovascular risk from school-age to adulthood.[51-53] Intima-media thickness and distensibility reflect structural and functional changes in the arteries, respectively.[54] Although previous studies strongly suggest that early-life weight growth patterns affect cardiovascular health in later life, it is still not known whether infant weight growth patterns or body mass trajectories across childhood are associated with markers of arterial health. Therefore, in **Chapter 2.3**, we assessed whether weight growth from infancy and across school-age is associated with markers of arterial health at 10 years. We observed that both higher peak weight velocity and BMI at adiposity peak were associated with higher carotid intima-media thickness at age 10 years. To date, our study was the first to report associations between infant weight growth velocity patterns and childhood carotid intima-media thickness. We also observed that infant peak weight velocity and BMI at

adiposity peak were inversely associated with carotid distensibility at age 10 years. However, these associations were fully explained by BMI at 10 years, which highlights the importance of weight management already in childhood. Higher BMI in childhood and adolescence has been associated, mostly using a cross-sectional design, with higher carotid intima-media thickness and lower distensibility.[51, 55-57] In line with previous studies, we observed that BMI across childhood was positively associated with carotid intima-media thickness and negatively associated with carotid distensibility at 10 years. However, the observed associations between BMI and carotid intima-media thickness were restricted to lean children. Thus, our results were more consistent for carotid distensibility than for carotid intima-media thickness, suggesting that suboptimal BMI in children may be associated with early functional changes of the carotids.

The mechanisms underlying the observed associations of body weight and growth measures across childhood with markers of arterial health in late childhood are largely unknown.[58] A plausible explanation may involve metabolic complications associated with obesity. Adipose cells are metabolically active and produce leptin that is a hormone that regulates appetite, body weight, and is involved in vascular physiology. Also, it has angiogenetic activity, increases oxidative stress in endothelial cells and promotes vascular cell calcification.[59] Atherosclerosis and arterial stiffening are distinct, but are synergistic processes that often coexist and share risk factors. Arterial stiffening activates pathophysiologic mechanisms involved in atherogenesis.[60] As our findings were more consistent for carotid distensibility than for carotid intima-media thickness, it suggests that functional changes may precede structural abnormalities.[61] Also, BMI is the sum of lean and fat mass index but cannot distinguish between these components. Lean mass is metabolically more active than fat mass and the main determinant of resting energy expenditure.[54] Lean mass thus increases oxygen demand, which requires a higher cardiac output subsequently increasing blood pressure.[62] High blood pressure has been linked to higher intima-media thickness and lower distensibility.[53, 63] Therefore, lean mass has been suggested to be a stronger determinant of cardiovascular structure and function than BMI in children.[62-64] Although more extreme values may be pathological, in healthy school-aged children, subtle differences in intima-media thickness and carotid distensibility may reflect normal adaptation to lean mass and blood pressure.[55]

Altogether, the findings of this thesis suggest that the associations of infant weight growth patterns and BMI across school-age with measures of arterial health are complex and might be different for intima-media thickness and distensibility. Also, the effect estimates of the observed associations were small and may not be relevant at an individual level. Yet, on a population-based level, our findings underline the importance of a healthy weight from infancy onwards.

Chemical exposures

Endocrine disrupting chemicals, such as phthalates and bisphenols, are adverse environmental factors that may affect childhood health.[65] Due to the widespread use of phthalate metabolites and bisphenols-related products, children can be highly exposed to these potential harmful chemicals.[66] Specifically, phthalates and bisphenols may permanently disrupt metabolic pathways, mainly through epigenetic and endocrine mechanisms, contributing to an adverse cardiovascular profile.[66, 67] High exposure to phthalate metabolites and bisphenols is increasingly reported to be associated with obesity, hypertension, insulin resistance, dyslipidemia, and cardiovascular disease among adults.[68, 69] Evidence among children is less consistent and mostly used a cross-sectional design.[70-73] Thus, in **Chapter 2.4**, we examined whether phthalate metabolites and bisphenols urinary concentrations at 6 years were associated with body fat measures and cardiovascular risk factors at 6 and 10 years, as well as with the change in these outcomes from 6 to 10 years. Since potential ethnicity-specific differences in the associations were previously reported, we excluded children with non-Dutch ethnicity.[71] Among 471 Dutch children, we observed that higher phthalate concentrations, specifically di-n-octyl phthalate (DNOP) metabolites, at 6 years were associated with higher BMI and an increased risk of overweight and obesity at 6 and 10 years while higher total bisphenols and bisphenol A (BPA) urinary concentrations were associated with a decrease in BMI from 6 to 10 years. Our findings were mostly in line with previous studies for the associations between phthalates and BMI, but not for bisphenols.[70, 74] In addition, besides the potential ethnicity-specific differences, previous studies suggested differences by sex, potentially due to estrogenicity of bisphenols and antiandrogenicity of some phthalates.[74] We did not observe a statistical interaction by child's sex, but our results might have been underpowered to detect differences by sex due to small sample size. The associations of phthalate metabolites and bisphenols exposure with cardiovascular outcomes during childhood have also been explored and showed inconsistent results.[72, 75-77] We observed that higher DNOP metabolites were associated with lower HDL-cholesterol and tended to be associated with higher systolic blood pressure and higher triglycerides while higher total bisphenols and BPA urinary concentrations tended to be associated with higher diastolic blood pressure and lower insulin in school-age children. However, these associations should be interpreted with caution since the effect estimates attenuated after multiple testing correction and some changed to non-significance.

The potential mechanisms underlying the associations of phthalate metabolites with overweight and an adverse cardiovascular profile in childhood might include the activation of peroxisome proliferator-activated receptors (PPARs) and the perturbation of the steroid and thyroid hormones system.[78-80] Activation of PPARs can increase lipid accumulation and release adipocyte-related hormones, increasing the susceptibility for the development of obesity.[80] Likewise, imbalance of steroid and thyroid hormones, which are critical for the maintenance of basal metabolism, may also have obesogenic effects.[79] Although not in line

with the findings of this thesis, similar mechanisms have been described for bisphenols. In addition, generalized increase in oxidative stress and subsequent endothelial dysfunction may account for the adverse effects of phthalates in the cardiovascular outcomes.[72]

Results from this thesis suggest that DNOP metabolites and bisphenols exposure may affect childhood BMI and cardiovascular profile. Although small, the observed effect estimates might be important on a population-based level, as children are widely exposed to these chemicals and overweight and adverse cardiovascular risk factors tend to track into poorer cardiovascular health later in life.

Main findings

- Maternal psychological distress and anxiety during pregnancy were associated with higher general and organ fat measures in offspring aged 10 years. Psychological distress, depression and anxiety were associated with higher heart rate among boys and maternal anxiety was also associated with higher triglycerides concentrations among girls.
- Peak weight velocity and body mass index at adiposity peak were positively associated with childhood carotid intima-media thickness. Body mass index across childhood was inversely associated with carotid distensibility at age 10 years.
- Higher DNOP metabolites urinary concentrations were associated with overweight and an adverse cardiovascular profile in childhood. Higher total bisphenols and bisphenol A were associated with a decrease in BMI from 6 to 10 years.

Childhood brain health

Maternal cardiovascular health

Poor cardiovascular health of mothers during pregnancy has been associated with pregnancy complications and long-term adverse outcomes for the offspring.[81, 82] As human brain development is a complex ongoing process that begins early in pregnancy, adverse exposures during this period may potentially alter fetal brain development, which may subsequently influence brain health in later life.[83, 84] Although previous results suggested a link between maternal obesity, hypertension and diabetes during pregnancy and adverse neurocognitive outcomes during childhood, studies performed thus far have mainly focused on maternal clinically manifest diseases and do not allow for clear conclusions about the associations of maternal cardiovascular risk factors with offspring brain structure.[85-89] Therefore, in **Chapter 3.1**, we examined whether maternal cardiovascular health factors, including BMI, gestational weight gain, blood pressure, and insulin, glucose, and lipids concentrations in early pregnancy are associated with childhood brain structure at 10 years of age. We observed that higher

maternal diastolic blood pressure in early pregnancy was associated with lower global white matter mean diffusivity (MD) in the offspring. Diffusion tensor imaging (DTI) was introduced as an unique magnetic resonance imaging (MRI) technique to assess microstructural properties of white matter integrity based on its ability to measure patterns of water diffusion in the brain.[90] Common parameters to represent white matter integrity from DTI include fractional anisotropy (FA), describing the degree of anisotropic diffusion, and mean diffusivity (MD), describing the average diffusion in all directions.[91, 92] While still limited, previous DTI studies suggest a link between neurodevelopmental disorders and cognitive abilities and differences in white matter microstructure among children and adolescents.[90, 92]. However, since in this thesis we focused on brain morphology and structural connectivity only, caution is warranted regarding potential functional implications of the observed associations.

In addition, we observed that lower maternal BMI and weight gain in early pregnancy tended to be associated with offspring smaller brain volumes. In line with our findings, previous studies suggested that not only maternal obesity, but also underweight is associated with poorer neurodevelopment outcomes in the offspring.[93, 94] Literature on the association between gestational weight gain and offspring neurodevelopment is limited and inconsistent. While some studies reported positive associations of gestational weight gain with cognitive development outcomes in the offspring, other studies showed no association or inverse association.[88, 94-96] In this thesis, differently from most previous studies, we focused specifically on weight gain in early pregnancy since it largely reflects maternal fat deposition, whereas gestational weight gain in mid and late pregnancy reflects growth of the fetus, placenta, and uterus. However, our results should be interpreted with caution as none of these associations remained statistically significant after multiple testing correction. We did not observe associations of maternal insulin, glucose, and lipids concentrations in early pregnancy with childhood brain structure. A possible explanation for our findings is that subtle differences in maternal metabolic risk factors before the onset of a clinically manifest disease may not trigger detectable structural changes in offspring brain.

Different mechanisms could explain the observed associations. A suboptimal placental perfusion due to high blood pressure in pregnancy may negatively affect ongoing neurodevelopmental processes that start prenatally and continue throughout childhood, such as axonal development or myelination, which can subsequently influence brain health in later life.[97] In addition, previous studies showed that altered HPA axis and immune system functioning were associated with both hypertensive pregnancy disorders and neurodevelopmental outcomes in offspring.[98-101]

Altogether, the findings of this thesis suggest that maternal diastolic blood pressure may, independently of socioeconomic and lifestyle family factors, be associated with differences in offspring white matter microstructure. Our results may be important from a developmental perspective since adverse exposures during pregnancy may potentially lead to long-term consequences for offspring brain development.

Growth patterns

The time from conception through 2 years of age has been recognized as a critical period of development.[102, 103] Adverse experiences occurring during this period may permanently influence brain structure and function.[104, 105] The existing literature to support this hypothesis is mainly focused on children born preterm or with low birth weight and suggested that those are at risk for suboptimal neurodevelopmental outcomes.[106-109] Although gestational age and weight at birth have been largely used as determinants of child health, they are merely the end point of fetal development and the starting point for infancy. During infancy, children born small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA) have different growth patterns.[110] Longitudinal growth patterns from fetal life until infancy may help to identify windows of vulnerability for brain development. Thus, in **Chapter 3.2**, we assessed whether early-life growth patterns are associated with childhood brain morphology. We observed that increased weight gain until the second and third trimester, birth, and ages 6, 12, and 24 months was associated with larger brain volumes, independently of growth during other age windows. Interestingly, the largest differences in brain volume were observed in relation to weight gain from late gestation to birth, whereas after 6 months of age the effect estimates were of smaller magnitude. Similarly, previous evidence suggested that late gestation and the first postnatal months represent a critical period of brain development, in which the human brain experiences a striking growth.[111] We also observed that, compared with children with normal fetal and infant growth, those who experienced fetal weight deceleration followed by infant catch-up growth had similar brain volumes at age 10 years. Moreover, higher peak weight velocity and BMI at adiposity peak were, independently of childhood BMI, associated with larger brain volumes. Similarly, previous observational studies suggested that faster weight gain during the first 2 years of life might be associated with benefits to neurocognitive functioning later in life.[112, 113] However, in this thesis we focused on brain structure only, and thus whether the observed associations link to neurocognitive outcomes should be further investigated.

The underlying mechanisms may include fetal programming adaptations and genetic predisposition. A suboptimal intrauterine environment may lead to fetal adaptive responses, such as circulatory redistribution, that negatively affect brain development.[114, 115] Also, previous studies have found that the genetic background of birth weight is also associated with head circumference at birth and in later life.[116-118]

The findings of this thesis suggest that fetal and infant weight growth are associated with brain morphology in school-age children. Understanding the association of early-life growth patterns with neurodevelopment is extremely important from a developmental and preventive perspective.

Childhood cardiovascular health

As a result of the childhood obesity epidemic, related cardiovascular risk factors, such as high blood pressure and adverse lipids profile are also increasing among children and adolescents.[6, 9] Cardiovascular risk factors are known to be associated with brain health in adults.[119, 120] Also, a growing body of studies suggests associations of obesity and adverse cardiovascular risk factors with smaller brain volumes and deficits in neurocognitive and executive functions in childhood and adolescence.[121-123] However, these studies have mainly focused on indirect measurements of adiposity, were of modest sample size and remain limited on evaluating the association of cardiovascular risk factors with brain structure. Therefore, in **Chapter 3.3**, among 3098 children aged 10 years, we investigated the associations of detailed body fat measures and cardiovascular risk factors, such as BMI, fat mass index, android fat mass percentage, blood pressure, and insulin, glucose, and lipids concentrations with brain structure. We observed that, compared to children with a normal weight, those with underweight have smaller brain volumes. Also, higher fat mass index and android fat mass percentage were associated with smaller brain volumes and lower white matter MD, respectively. Interestingly, our findings are in line with a previous study showing inverse U-shaped associations of childhood BMI with less cortical gyrification, potentially suggesting that both lower and higher BMI might be associated with different brain development trajectories during childhood.[124] In this thesis, none of the other cardiovascular risk factors were associated with brain structure after adjustment for multiple testing. Conversely, previous results from the same cohort used in this thesis, the Generation R Study, showed an association between higher diastolic blood pressure and lower cognitive function in children aged 6 years.[7] A possible explanation for this divergence in findings is that even though subclinical changes in cardiovascular risk factors may not be enough to trigger brain structural differences yet, it might potentially affect its function.

The potential mechanisms underlying the observed associations may include inflammatory and endocrine changes. Increased inflammation, hormonal changes, and oxidative stress have been associated to endothelial dysfunction and vascular reactivity negatively affecting brain structure and function.[125, 126] Previous studies suggested a link between childhood obesity and low-grade inflammation, increased C-reactive protein, and alterations in endocrine processes.[127, 128] Likewise, a study among anorexic adolescents showed that underweight was related to higher levels of interleukin-6, apolipoprotein-B and global endocrine dysregulation.[129]

Altogether, the results of this thesis suggest that body weight and body fat distribution relate to children's brain morphology and white matter microstructure at the age of 10 years. On a population-based level, our findings highlight the importance of a healthy weight already early in life.

Main findings

- Maternal diastolic blood pressure in early pregnancy was associated with differences in white matter microstructure in offspring aged 10 years.
- Increased weight gain during the fetal period and the first 2 years of life was associated with larger brain volumes later in childhood. Compared with children with normal fetal and infant growth, those who experienced fetal weight deceleration but showed postnatal catch-up growth had similar brain volumes at age 10 years.
- In children aged 10 years, body weight and body fat distribution, but not other cardiovascular risk factors, were associated with brain morphology and white matter microstructure.

METHODOLOGICAL CONSIDERATIONS

Specific strengths and limitations for the studies presented in this thesis are described in **Chapter 2** and **Chapter 3**. In the following paragraphs, general methodological considerations regarding selection bias, information bias, confounding and causality are discussed.

Selection bias

Selection bias might occur when the association between the exposure and outcome of interest is different in subjects who participate in the study and in those who were eligible for the study, but do not participate.[130] Consequently, the obtained results may not be representative for the population of interest. For example, in the Generation R study, of all eligible children at birth, 61% participated at baseline.[131] As compared to the general population in Rotterdam, participants from the Generation R study were more often from European ethnic background, had a higher socio-economic status and fewer adverse birth outcomes, suggesting a selection towards a healthier study population. As a result, this selection may have led to lower prevalence rates of maternal psychological distress, and associated risk factors, and subsequently reduced statistical power. In addition, it may affect the generalizability of our findings to other, less healthy populations. However, previous analyses in cohort studies demonstrated that the studied associations were not strongly influenced by selection bias at baseline, therefore it seems unlikely that the results of this thesis are biased due to selective non-response at baseline.[132, 133]

Selection bias is also possible due to selective loss to follow-up and, in this case, may occur if the association between the exposure and outcome of interest is different between those included in the analyses and those lost to follow-up. In the studies presented in this thesis, we used data from the follow-up at 6 and 10 years of age.[131] At the age of 6 and 10 years, children were invited to participate in detailed body fat and cardiovascular follow-up mea-

sures, as well as neuroimaging assessment. Compared to the total follow-up group, a lower percentage of children participated in biological sample procedures at 6 years, and in the MRI measurements of the brain and organ fat at 10 years, which was mainly due to non-consent. Mothers of children who did visit the research centre in the follow-up evaluations were more often of Dutch origin, higher educated and, frequently, had healthier lifestyle habits than those who did not participate in follow-up visits. This selective loss to follow-up towards a healthier population may have biased our observed effect estimates, although it is difficult to quantify the extent.

Information bias

Information bias refers to a bias in an estimate arising from measurement errors or misclassification.[130] In this thesis, fetal weight was estimated using the data obtained by second and third trimester ultrasonography which might be prone to measurement error and may have influenced our findings.[134] Also, we relied on a single-spot urinary measurement of phthalate metabolites and bisphenols as an estimate of exposure. Although it has been suggested that a single urine sample for phthalate concentrations reasonably reflects exposure for up to 3 months, both phthalate metabolites and bisphenols have short biological half-lives.[135] Thus, measurement error may have led to underestimation of the effect estimates. In addition, we relied on hands-on assessments of body composition and cardiovascular health, such as weight, height, head circumference and blood pressure, which might have greater measurement error and be less accurate, but on the other hand be easier and cheaper to obtain in large epidemiological studies. High accuracy and reproducibility have been reported for dual energy X-ray absorptiometry, ultrasound, and MRI techniques that were also used in this thesis.

Misclassification can be non-differential or differential. Non-differential misclassification is a random error that occurs unrelated to the exposure or outcome status, and may lead to an underestimation of the true effect. Differential misclassification is a non-random error that occurs when the exposure is misclassified and this is related to the outcome status, and vice-versa, which is more problematic as it may lead to an over- or underestimation of the true effect. In this thesis, information on exposures and outcomes was prospectively obtained by maternal questionnaires, physical and ultrasound examinations, biological samples, and MRI data.[131] In particular, exposure data used in our studies were collected before assessment of the outcomes, the data collectors were blinded to the exposure status when assessing the outcomes, and parents as well as data collectors were unaware of the specific research questions addressed in this thesis. Thus, differential misclassification seems unlikely, but non-differential misclassification might have occurred. In the studies included in this thesis, information on psychological distress during pregnancy was self-reported. A previous study suggested that women with certain psychiatric disorders tend to underestimate their own psychological problems, and this may lead to an underestimation of the observed effects.[136]

However, high reliability and validity of the Brief Symptom Inventory (BSI), which was the questionnaire used to access psychological distress in our studies, have been previously reported.[137] In addition, as the fasting time before blood sampling was limited to 30 minutes we consider that our studies relied on non-fasting samples. This may have resulted in non-differential misclassification. However, previous studies reported that semi-fasted insulin resistance is moderately correlated with fasting values and that non-fasting lipids are superior to fasting in accurately predicting the risk of cardiovascular disease later in life.[138, 139]

Confounding

A confounding factor is a variable associated with both exposure and outcome that explains all or part of the association and may not be an intermediate variable in the causal pathway between the exposure and the outcome.[130] Adjustment for confounding is needed to prevent biased effect estimates leading to either under- or overestimation of the observed effect. In this thesis, to account for confounding, we adjusted all analyses for multiple potential confounders. We selected confounders based on literature, their associations with the exposures and outcomes, and by observing a >10% change in effect estimates when adding the confounder to the basic model.[140] In some of our studies, effect estimates attenuated after adjustment for potential confounders, which indicates that the observed associations may be partly explained by these variables. Although in our studies we collected information on many potential confounding variables, residual confounding might still be present, as in any observational study. In addition, many of the potential confounders were derived from self-reported data, and therefore misclassification might have occurred, contributing to residual confounding.

Causality

The observational design of all studies reported in this thesis does not allow to conclude causality in the assessed associations. Causality between exposures and outcomes can be determined by the Bradford Hill criteria, which include strength, consistency, specificity, temporality, dose-response relationship, biological plausibility, coherence, experiment and analogy.[141] Overall, our effect sizes were relatively small. Although larger effect sizes are more likely to be causal, weak associations may also be causal. Most of our findings were consistent with previous literature, and the majority of the studies in this thesis had a longitudinal design, supporting the temporality between exposures and outcomes. In the cross-sectional study on child cardiovascular risk factors and brain structure at the same age, we could not draw conclusions about the direction of effects because it is uncertain whether the exposures occurred before the outcomes. Although not explored in all studies presented in this thesis, we did observe a tendency for dose-response effects for maternal stress, depression and anxiety with childhood adiposity. We observed stronger associations with childhood adiposity measures when mothers reported severe rather than moderate stress, depression,

or anxiety, however, the effect estimates were not statistically significant. This may be partly explained by a limited statistical power since a low number of women reported severe levels of stress. Additionally, several potential biological mechanisms for the observed associations have been described, and our findings were coherent with animal studies. The specificity, experiment and analogy criteria were not addressed in this thesis. Although the studies in this thesis were not designed to address the causality of these associations, our observational studies seem to provide some evidence for causal relationships based on the Bradford Hill criteria.

FUTURE RESEARCH

Although findings from this thesis suggest that lifestyle factors, growth and chemicals exposures may affect childhood cardiovascular and brain health, the following issues remain to be addressed in future studies.

Assessment of exposures

More detailed assessment of the exposures of interest might provide further insight in the studied associations. In this thesis, information on maternal psychological distress during pregnancy was obtained through the Brief Symptom Inventory (BSI) that is a self-report questionnaire.[137] Although previous studies reported good reliability and validity of the BSI in identifying women with symptoms of overall distress, depression and anxiety, the BSI refers to the previous 7 days only and was measured once in mid-pregnancy. Self-reported information of maternal stress may result in underreporting of psychological symptoms and subsequent underestimation of observed effects. Ideally, future studies should take into account both self-reported stress and clinical measures, such as cortisol levels and heart rate to have perceived and objective measures of stress. Also, studies with repeated measurements of maternal psychological distress broadens the understanding of the intensity and persistence of psychological symptoms during pregnancy. For the growth analyses, the use of fetal and infant weight measurements at 6 different time points from 2nd trimester until 24 months of age enabled us to construct and study 9 different growth patterns. Also, additional analysis using peak weight velocity, and BMI at adiposity peak provided more insight into growth physiology in our study. Future studies should consider a change in perspective, highlighting growth patterns instead of single time point measurements, such as birth weight, to evaluate children at risk for adverse neurodevelopment outcomes. For the chemicals exposures, similarly to our study, most previous studies investigating the effects of phthalate metabolites and bisphenols have depended on concentrations measured in 1 or 2 spot urine samples. However, these chemicals are characterized by a brief half-life and are excreted in urine after only 1 or 2 days.[142] This indicates a larger variability within each individual as a consequence of variable contact with exposure sources.

To increase accuracy, future studies should consider carrying out several repeated exposure measurements on each individual. Finally, for the maternal and childhood cardiovascular risk exposures we relied on single measurements performed at the same research visit. Studies with more detailed and repeated cardiovascular measurements will allow to early distinguish between physiological and pathological hemodynamic adaptations.

Assessment of outcomes

Childhood cardiovascular outcomes studied in this thesis included body composition, blood pressure, lipids, and insulin levels at 6 and 10 years. Also, information on carotid intima-media thickness and organ fat was available at 10 years. We used MRI, which is considered the gold standard technique for the measurement of intra-abdominal and organ fat deposition, at 10 years.[143] Since organ fat outcomes are related to various cardio-metabolic risk factors and track from childhood into adulthood, future studies should measure these body fat compartments at younger ages to obtain further insight into its development and health consequences. Also, we used ultrasound measurement of the carotid intima-media thickness that is considered a valid marker for cardiovascular risk allowing assessment of atherosclerotic changes at a very early stage.[144] However, the heterogeneity of techniques of scanning, measurement and interpretation have been interfering with the comparison of intima-media thickness values, especially for the paediatric age group. Additional measurements of childhood cardiovascular development might provide further insight into the underlying mechanisms linking early life exposures to cardiovascular outcomes in later life. Measures of microvasculature, such as retinal vascular imaging, as well as more refined cardiac measures obtained by MRI in children might be of interest to gain more knowledge about the cardiovascular development. Brain development outcomes included high-resolution structural and diffusion tensor MRI sequences. The studies in this thesis primarily focused on brain morphology at age 10 years. However, the human brain development starts soon after conception and continues across childhood and early adulthood. Studies examining neuroimaging outcomes at earlier and later ages might broaden the understanding on the various facets of brain development and give more insight into the identification of abnormal neurobiological processes. Future studies should investigate the associations of maternal and childhood cardiovascular health with repeated assessments of brain morphology. Finally, most neuroimaging studies examine a single modality at a time, for example, morphological MRI or DTI. As the underlying mechanism of adverse neurodevelopment outcomes is likely highly complex, the integration of multimodal neuroimaging techniques is needed.

Causality

Although we performed an extensive adjustment for potential confounding factors, the causality of the observed associations cannot be inferred due to the observational design of the included studies. Randomized controlled trials (RCT) are the gold standard study

design to establish causality. However, randomized exposure to externally regulated stress in pregnancy or cardiovascular health factors in mothers and children without a medical indication, is not ethically feasible. Instead, experimental interventions promoting psychological wellbeing and lifestyle modifications during pregnancy and in early childhood, may provide more insight into the potential causal association of these exposures with cardiovascular and brain health later in life. For example, studies have shown that prenatal mindfulness-based interventions are associated with reduced maternal perceived stress, anxiety, and depression, which could help promote a healthy pregnancy and in utero environment.[145] Also, long-term follow-up studies of participants in ongoing or completed randomized controlled trials that aim to improve diet and physical activity in pregnant women and children with obesity or increased cardiovascular risk are important.[146, 147] Ideally, these trials would provide an unique opportunity to examine whether cardiovascular risk factors are causally related to childhood brain development. A previous review including 18 RCTs already suggested that physical activity, diet and some behavioural interventions might improve cognition and school achievement in children and adolescents with obesity.[148] However, the effect of these interventions on childhood brain structure remains to be explored. Considering the findings of this thesis that suggest that promoting a healthy maternal mental state as well as cardiovascular profile during pregnancy and early childhood might be of greater importance for later cardiovascular and brain outcomes, randomized controlled trials focused on the early-life period, specifically preconception, early pregnancy and infancy, are needed.

Further, sibling comparison studies and Mendelian randomization studies can also be used to assess causality.[149, 150] Sibling comparison studies allow control for maternal genotype and environmental factors that are shared among siblings. However, this study design might be limited to assess causality due to differences in major exposures of interest and other lifestyle-related characteristics between siblings.[150] Finally, large Mendelian randomization studies that use genetic variants, known to be robustly associated with the exposure of interest and not affected by confounding, as an instrumental variable are needed to provide further insight into the causality of the associations.

IMPLICATIONS FOR CLINICAL PRACTICE AND POLICY

Early-life exposures might affect childhood cardiovascular and brain health in the short and long term. Findings from this thesis suggest that maternal psychological distress during pregnancy and early childhood growth patterns and chemicals exposure are associated with cardiovascular health later in childhood. The effect estimates for the observed associations were small, however, might contribute to the burden of childhood obesity and related cardiovascular risk factors at a population level. In addition, our findings suggest that fetal and infant growth patterns as well as maternal and childhood cardiovascular risk factors are

associated with brain structure in 10-year-old children. The observed effect estimates were small to moderate, but might be of interest from a developmental and preventive perspective since they may help to identify windows of vulnerability for brain development. Altogether, if proved causal, the findings of these thesis highlight the importance of the first 1,000 days of life, time from conception through 2 years of age, as a critical period of development in which the foundations for a healthy growth is established. As mentioned previously, the studies presented in this thesis are based on observational data, which limits the identification of causality and translation of the findings to clinical practice. Despite this, the results of this thesis have some important clinical implications.

Preventive strategies or interventions focused on the preconception period or early pregnancy should be implemented. For most women, pregnancy is a natural moment to voluntarily seek medical support and improve health, which makes it a potentially interesting moment to lifestyle changes. In particular, based on our findings, maternal psychological distress and anxiety during pregnancy were associated with higher childhood general and organ fat measures as well as higher heart rate among boys and higher triglycerides among girls. Our findings also suggest a link between maternal BMI, gestational weight gain and diastolic blood pressure and offspring brain structure. Thus, it is essential to promote knowledge about the importance of a healthy physical and mental state in women of reproductive age. Health care providers involved in obstetric care should identify and advise women at risk for developing psychological distress and cardiovascular symptoms early in pregnancy or ideally, before conception, to potentially improve offspring health outcomes.

Furthermore, infancy and early childhood are also essential periods for implementation of preventive lifestyle interventions. Specifically, our findings suggest that infant peak weight velocity and BMI at adiposity peak were associated with carotid intima-media thickness and brain volumes at age 10 years. Thus, this highlights the importance of informing families about the benefits of a healthy weight and body composition that might impact the health of their child later in life. Health care providers including general practitioners and pediatricians, should also be aware of this relation and aim to prevent obesity in childhood. As schools play an important role in providing nutrition and health education, promoting physical activity and a healthy environment for students should also be a target of intervention.

CONCLUSION

Findings presented in this thesis suggest that early-life determinants, such as maternal psychological distress, growth patterns, chemicals exposure and cardiovascular risk factors from early pregnancy onwards, are associated with childhood cardiovascular and brain health outcomes. The observed associations are relatively small, but may be important for the burden of cardiovascular and neurodevelopment disorders on a population level.

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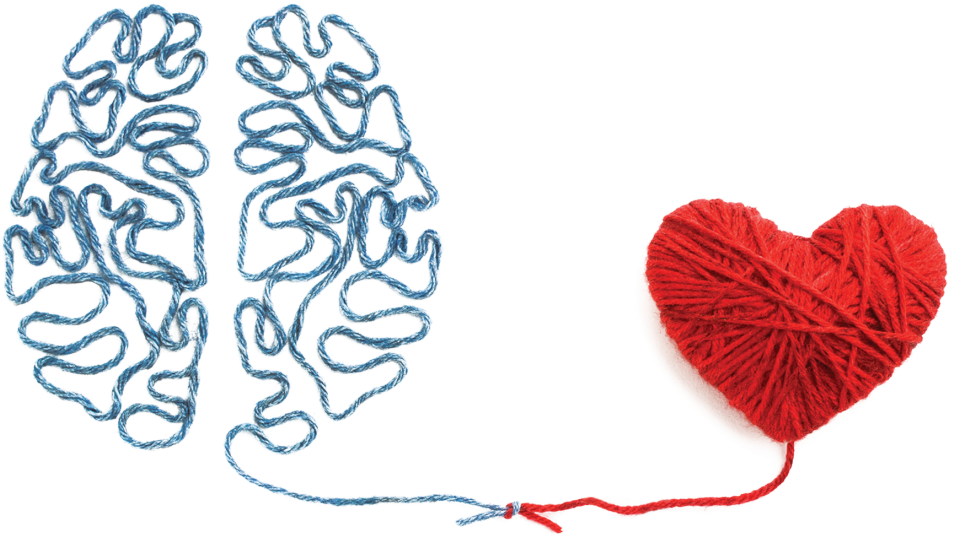


Chapter 5

Summary
Samenvatting

SUMMARY

Here, a general overview of the thesis is provided. **Chapter 1** provides the background, hypothesis, aims and design for the studies presented in this thesis. Cardiovascular and neurodegenerative disorders are major causes of mortality and morbidity in the general adult population worldwide. Cardiovascular and brain health are closely related and share some common risk factors such as obesity, hypertension, diabetes and dyslipidemia. It has been proposed that adverse cardiovascular and neurodevelopmental outcomes in childhood and adulthood may originate in early life. Assessing the influence of early-life determinants on detailed cardiovascular and brain outcomes measures during childhood presents an opportunity to evaluate whether adverse early-life exposures may trigger manifestations of poorer health before the onset of clinically manifest diseases. Therefore, the general aim of this thesis was to assess the associations of early-life determinants, in particular psychological distress, growth, lifestyle and chemical exposures, with childhood cardiovascular and brain health. The studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.

Chapter 2 of this thesis focuses on childhood cardiovascular health. In **Chapter 2.1** and **Chapter 2.2**, we examined the associations of maternal psychological distress during pregnancy with offspring body fat measures and cardiovascular risk factors at 10 years. We observed that maternal psychological distress and anxiety during pregnancy were associated with higher offspring general and organ fat measures in childhood. Psychological distress, depression and anxiety were associated with higher heart rate among boys and maternal anxiety was also associated with higher triglycerides concentrations among girls. These findings suggest that maternal psychological distress during pregnancy may influence body fat, and in a sex-specific manner, cardiovascular profile, during childhood. In **Chapter 2.3**, we evaluated whether weight growth from infancy and across school-age is associated with markers of arterial health at 10 years. We found that peak weight velocity and body mass index (BMI) at adiposity peak were positively associated with childhood carotid intima-media thickness. BMI across childhood was inversely associated with carotid distensibility at 10 years. The associations of infant weight growth patterns and BMI across school-age with measures of arterial health are complex and might be different for intima-media thickness and distensibility. Although the observed effect estimates were small and may not be relevant at an individual level, on a population level, our findings underline the importance of a healthy weight from infancy onwards. Next, we assessed the associations of phthalate metabolites and bisphenols urinary concentrations at 6 years with body fat measures and cardiovascular risk factors at 6 and 10 years, as well as with the change in these outcomes from 6 to 10 years (**Chapter 2.4**). We observed that higher di-n-octyl phthalate metabolites urinary concentrations were associated with overweight and an adverse cardiovascular profile in childhood. Higher total

bisphenols and bisphenol A urinary concentrations were associated with a decrease in BMI from 6 to 10 years. These findings suggest that adiposity and cardiovascular profile in school-aged children may be influenced by phthalate metabolites and bisphenols exposure. Our results are important from a developmental perspective since children are widely exposed to these chemicals and overweight and adverse cardiovascular risk factors tend to track into poorer cardiovascular health later in life.

Chapter 3 of this thesis focuses on childhood brain health and its link with childhood cardiovascular health. In **Chapter 3.1**, we investigated whether maternal cardiovascular health factors, including BMI, gestational weight gain, blood pressure, and insulin, glucose, and lipids concentrations in early pregnancy, are associated with childhood brain structure at 10 years. We found that higher maternal diastolic blood pressure in early pregnancy was associated with lower global white matter mean diffusivity in offspring aged 10 years. Lower maternal BMI and weight gain in early pregnancy tended to be associated with offspring smaller brain volumes. We did not observe associations of maternal insulin, glucose, and lipids concentrations in early pregnancy with childhood brain structure. These findings suggest that an adverse cardiovascular health profile during early pregnancy may potentially lead to long-term consequences for offspring brain development. In **Chapter 3.2**, we explored the associations of early-life growth with childhood brain morphology at 10 years. Increased weight gain during the fetal period and the first 2 years of life was associated with larger brain volumes later in childhood. Compared to children with normal fetal and infant growth, those who experienced fetal weight deceleration but showed postnatal catch-up growth had similar brain volumes at 10 years. Our results suggest that fetal and infant weight growth patterns are associated with brain morphology in school-age children. These findings warrant future studies to consider a change in perspective, highlighting growth patterns instead of single time point measurements, such as birth weight only. This will support the identification and evaluation of children that may potentially be at a higher risk for adverse neurodevelopment outcomes. In **Chapter 3.3**, we investigated the associations of detailed body fat measures and cardiovascular risk factors, such as BMI, fat mass index, android fat mass percentage, blood pressure, and insulin, glucose, and lipids concentrations with brain structure at 10 years. We observed that body weight and body fat distribution, but not other cardiovascular risk factors, were associated with brain morphology and white matter microstructure in 10-year-old children. On a population level, our findings highlight the importance of a healthy weight early in life for a healthy brain development.

Finally, in **Chapter 4**, a general discussion of all studies included in this thesis, suggestions for future research and implications for clinical practice and policy are presented.

SAMENVATTING

Hier is de algemene samenvatting van het proefschrift beschreven. **Hoofdstuk 1** omschrijft de achtergrond, hypothese, doelstellingen en opzet voor de studies die in dit proefschrift worden gepresenteerd. Wereldwijd, zijn cardiovasculaire en neurodegeneratieve aandoeningen de belangrijkste oorzaken van mortaliteit en morbiditeit in volwassenen. Cardiovasculaire gezondheid en gezondheid van de hersenen zijn nauw verwant en delen enkele gezamelijke risicofactoren zoals obesitas, hypertensie, diabetes en dyslipidemie. Er is gesuggereerd dat nadelige cardiovasculaire uitkomsten en hersenontwikkeling in de kindertijd en op volwassen leeftijd hun oorsprong kunnen vinden in het vroege leven. Het onderzoeken van de invloed van vroege determinanten op gedetailleerde cardiovasculaire uitkomsten en hersenontwikkeling in de kindertijd biedt een mogelijkheid om te bepalen of ongunstige vroege blootstellingen kunnen leiden tot het ontstaan van ziekten, al voor dat er klinische manifestaties zijn. Het algemene doel van dit proefschrift is het onderzoeken van de relatie tussen vroeg detecteerbare determinanten, in het bijzonder psychologische stress, groei, leefstijl factoren en blootstelling aan chemicaliën, in relatie tot cardiovasculaire uitkomsten en hersenontwikkeling bij kinderen. De studies gepresenteerd in dit proefschrift zijn onderdeel van de Generation R Studie, een prospectief populatie gebaseerd cohortonderzoek welke gestart is vanaf de vroege zwangerschap en waarin de deelnemers gevolgd worden tot hun jong volwassenheid in Rotterdam, Nederland.

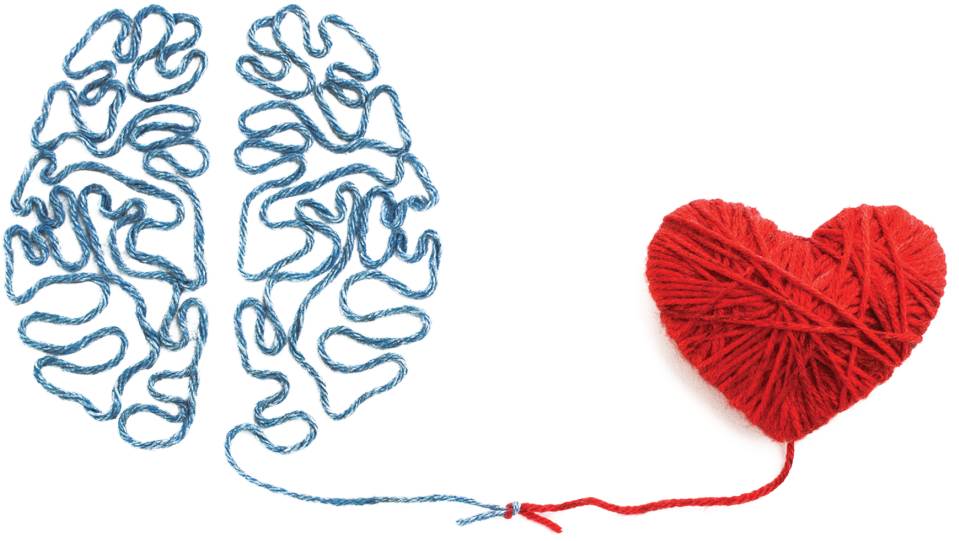
Hoofdstuk 2 van dit proefschrift richt zich op de cardiovasculaire gezondheid van kinderen. In **Hoofdstuk 2.1** en **Hoofdstuk 2.2** onderzochten we de associaties van psychologische stress bij moeders tijdens de zwangerschap met lichaamsvetmetingen in kinderen en cardiovasculaire risicofactoren op de leeftijd van 10 jaar. We observeerden dat psychologische stress en angst van de moeder tijdens de zwangerschap geassocieerd waren met hogere algemene en orgaanvetmetingen in de kinderen. Psychische stress, depressie en angst waren geassocieerd met een hogere hartslag bij jongens en angst van de moeder was ook geassocieerd met hogere triglyceridenconcentraties bij meisjes. Deze bevindingen suggereren dat psychologische stress van de moeder tijdens de zwangerschap het lichaamsvet bij hun kinderen kan beïnvloeden, en op een sekse-specifieke manier invloed heeft op het cardiovasculaire profiel tijdens de kindertijd. In **Hoofdstuk 2.3** evalueerden we of gewichtstoename vanaf de kindertijd tot aan de schoolleeftijd geassocieerd is met markers van arteriële gezondheid na 10 jaar. We ontdekten dat de piekgewichtssnelheid en de body mass index (BMI) positief geassocieerd waren met de dikte van de halsslagader in de kindertijd. BMI tijdens de kindertijd was omgekeerd evenredig geassocieerd met de vaatwandstijfheid van de halsslagader op de leeftijd van 10 jaar. De associaties tussen de groeipatronen gedurende de kindertijd en het BMI in de schoolgaande leeftijd en de metingen van arteriële gezondheid zijn complex en kunnen verschillen in de dikte en uitzetbaarheid van de intima-media. Hoewel de geschatte effecten klein waren en mogelijk niet relevant zijn op individueel

niveau, onderstrepen onze bevindingen toch het belang van een gezond gewicht op populatieniveau vanaf de kindertijd. Daarnaast onderzochten we de associaties van ftalaatmetabolieten en bisfenolconcentraties in de urine na 6 jaar met lichaamsvetmetingen en cardiovasculaire risicofactoren op 6 en 10 jaar, evenals de verandering in deze uitkomsten van 6 tot 10 jaar (**Hoofdstuk 2.4**). We hebben waargenomen dat hogere urineconcentraties van di-n-octylftalaatmetabolieten geassocieerd waren met overgewicht en een ongunstig cardiovasculair profiel in de kindertijd. Hogere totale bisfenolen en bisfenol A-concentraties in de urine waren geassocieerd met een afname in BMI van 6 tot 10 jaar. Deze bevindingen suggereren dat adipositas en het cardiovasculair profiel van schoolgaande kinderen kunnen worden beïnvloed door blootstelling aan ftalaatmetabolieten en bisfenolen. Onze resultaten zijn belangrijk vanuit een ontwikkelingsperspectief, aangezien kinderen op grote schaal worden blootgesteld aan deze chemicaliën. Daarnaast kunnen overgewicht en ongunstige cardiovasculaire risicofactoren in de kindertijd het risico op een slechtere cardiovasculaire gezondheid op latere leeftijd vergroten.

Hoofdstuk 3 van dit proefschrift richt zich op de hersenontwikkeling bij kinderen en het verband met de cardiovasculaire gezondheid van kinderen. In **Hoofdstuk 3.1** hebben we onderzocht of maternale cardiovasculaire gezondheidsfactoren, waaronder BMI, gewichtstoename tijdens de zwangerschap, bloeddruk en insuline-, glucose- en lipidenconcentraties in de vroege zwangerschap, geassocieerd zijn met de hersenstructuur van de kindertijd na 10 jaar. We vonden dat een hogere maternale diastolische bloeddruk in het begin van de zwangerschap geassocieerd was met een lagere globale gemiddelde diffusiviteit van de witte stof bij kinderen van 10 jaar oud. Een lager maternaal BMI en gewichtstoename in het begin van de zwangerschap waren voornamelijk geassocieerd met kleinere hersenvolumes van het kind. We vonden geen associaties van maternale insuline-, glucose- en lipidenconcentraties in de vroege zwangerschap met de hersenstructuur in kinderen. Deze bevindingen suggereren dat een ongunstig cardiovasculair gezondheidsprofiel tijdens de vroege zwangerschap mogelijk kan leiden tot langetermijngevolgen voor de hersenontwikkeling van het kind. In **Hoofdstuk 3.2** hebben we de associaties tussen groei in het vroege leven en de hersenstructuur van kinderen op de leeftijd van 10 jaar onderzocht. Een verhoogde gewichtstoename tijdens de foetale periode en de eerste 2 levensjaren werd geassocieerd met grotere hersenvolumes in de kindertijd. Vergeleken met kinderen met een normale groei in de zwangerschap en in de eerste 2 levensjaren, hadden kinderen met foetale gewichtsvertraging en met een postnatale inhaalgroei vergelijkbare hersenvolumes op de leeftijd van 10 jaar. Onze resultaten suggereren dat groeipatronen in het vroege leven geassocieerd zijn met hersenmorfologie bij schoolgaande kinderen. Deze bevindingen moedigen toekomstige studies aan om een verandering in perspectief te overwegen, waarbij groeipatronen worden benadrukt in plaats van metingen op één tijdstip, zoals bijvoorbeeld alleen het geboortegewicht, om kinderen te identificeren en te evalueren die mogelijk een hoger risico lopen op nadelige hersenontwikkeling. In **Hoofdstuk 3.3** onderzochten we de associaties van gede-

tailleerde lichaamsvetmaten en cardiovasculaire risicofactoren, zoals BMI, vetmassa-index, androïde vetmassapercentage, bloeddruk en insuline-, glucose- en lipidenconcentraties in kinderen met hersenstructuur op de leeftijd van 10 jaar. We zagen dat lichaamsgewicht en lichaamsvetverdeling, maar niet andere cardiovasculaire risicofactoren, gerelateerd waren met hersenvolumes en de witte stof microstructuur in 10-jarige kinderen. Onze bevindingen benadrukken, op populatieniveau en al vroeg in het leven, het belang van een gezond gewicht voor een gezonde hersenontwikkeling.

Ten slotte beslaat **Hoofdstuk 4**, de algemene discussie van alle studies tezamen in dit proefschrift, en worden suggesties voor toekomstig onderzoek en implicaties voor de klinische praktijk en het beleid besproken.



Chapter 6

List of publications
PhD portfolio
About the author
Words of gratitude

LIST OF PUBLICATIONS

Silva CCV, Vehmeijer FOL, El Marroun H, Felix JF, Jaddoe VWV, Santos S. Maternal psychological distress during pregnancy and childhood cardio-metabolic risk factors. *Nutr Metab Cardiovasc Dis. 2019;29(6):572-9.*

Vehmeijer FOL, **Silva CCV**, Derks IPM, El Marroun H, Oei EHG, Felix JF, Jaddoe VWV, Santos S. Associations of maternal psychological distress during pregnancy with childhood general and organ fat measures. *Child Obes. 2019;15(5):313-322.*

Silva CCV, Jaddoe VWV, Sol CM, El Marroun H, Martinez-Moral MP, Kannan K, et al. Phthalate and Bisphenol Urinary Concentrations, Body Fat Measures, and Cardiovascular Risk Factors in Dutch School-Age Children. *Obesity (Silver Spring). 2021;29(2):409-17.*

Silva CCV, Jaddoe VWV, Muetzel RL, Santos S, El Marroun H. Body fat, cardiovascular risk factors and brain structure in school-age children. *Int J Obes (Lond). 2021;45(11):2425-31.*

Silva CCV, El Marroun H, Sammallahti S, Vernooij M, Muetzel RL, Santos S, Jaddoe VWV. Patterns of fetal and infant growth and brain morphology at age 10 years. *JAMA Netw Open. 2021 Dec 1;4(12):e2138214.*

Monasso GS, **Silva CCV**, Santos S, Gaillard R, Felix JF, Jaddoe VWV. Infant weight growth patterns, childhood BMI, and arterial health at age 10 years. *Obesity (Silver Spring). 2022;30(3):770-8.*

Silva CCV, Santos S, Muetzel RL, Vernooij M, van Rijn BB, Jaddoe VWV, El Marroun H. Maternal cardiovascular health in early pregnancy and childhood brain structure. *J Am Heart Assoc. 2022;11(19):e026133.*

Gonçalves R, Wiertsema CJ, **Silva CCV**, Monasso GS, Gaillard R, Steegers EAP, Santos S, Jaddoe VWV. Associations of Fetal and Infant Growth Patterns with Early Markers of Arterial Health. *JAMA Netw Open. 2022 Jun 1;5(6):e2219225.*

Monasso GS, Santos S, **Silva CCV**, Geurtsen ML, Oei EHG, Gaillard R, Felix JF, Jaddoe VWV. Body fat, pericardial fat, liver fat and arterial health at age 10 years. *Pediatr Obes. 2022; doi: 10.1111/ijpo.12926.*

Beunders VAA, Koopman-Verhoeff E, Vermeulen MJ, **Silva CCV**, Jansen PW, Luik AI, Reiss IKM, Joosten KFM, Jaddoe VWV. Fetal and infant growth patterns, sleep and 24-hour activity rhythms. A population-based prospective cohort study in school-age children. *Submitted*

Defina S, **Silva CCV**, Muetzel RL, Cecil CAM, Tiemeir H, Felix J, Jaddoe VWV. Arterial health and brain morphology in early adolescence: A population-based study. *Manuscript in progress.*

PHD PORTFOLIO

Name PhD candidate	Carolina Costa Vicente Silva
Erasmus MC Department	Pediatrics, Erasmus MC, Rotterdam
Medical school	Federal University of the State of Rio de Janeiro (2008-2014)
Research school	Netherlands Institute for Health Sciences (NIHES), Rotterdam (2019-2021)
PhD Period	June 2018 – June 2022
Promotors	Prof. Dr. V.W.V. Jaddoe, Prof. Dr. H. El Marroun
Co-promotor	Dr S. Santos

PhD Training	Year	Workload (ECTS)[1]
Master of Science in Clinical Epidemiology, NIHES, Rotterdam	2019-2021	70,6
Common core		
Study Design	2020	
Biostatistical Methods I: Basic Principles	2019	
Biostatistical Methods II: Classical Regression Models	2019	
Principles of Research in Medicine and Epidemiology	2019	
Research project	2021	
Introduction to Medical Writing	2019	
Required		
Clinical Translation of Epidemiology	2020	
Clinical Epidemiology	2020	
Repeated Measurements in Clinical Studies	2021	
Principles in Cause Inference	2020	
Methods of Public Health Research	2019	
Health Economics	2019	
Introduction to Global Public Health	2019	
The Practice of Epidemiologic Analysis	2019	
Fundamentals of Medical Decision Making	2019	
Advances in Clinical Epidemiology	2020	
Elective courses		
Methods of Health Services Research	2020	
Causal Mediation Analysis	2020	
Advanced topics in Decision-making in Medicine	2021	
Causal Inference	2020	
Missing Values in Clinical Research	2021	
Courses		
STeLa Workshop	2020	0,3
Scientific Integrity for PhD students, Erasmus MC, Rotterdam	2021	0,3

Intervision	2021	0,4
Seminars and workshops		
Research meetings Generation R Study	2018-2022	1,0
Maternal and Child Health meetings	2018-2022	4,0
Neurodevelopment group meetings	2021-2022	1,0
Behavior group meetings	2022	0,3
Conferences and presentations[2]		
Sophia Research Day, Rotterdam *Oral presentation*	2019	1,4
Weon, Annual Epidemiological Congress, Amsterdam *Online presentation*	2021	0,7
Sophia Research Day, Rotterdam *Online presentation*	2021	0,7
Prizes and awards		
'Big Data' – prize for the best abstract, Sophia Research Day	2021	
Teaching activities – supervising students		
I. Fontes Marques, PhD student: carotid intima-media thickness ultrassound scoring	2021	1,0
Other activities		
Coordinate the carotid IMT measurements at 10 years	2018-2019	
Peer review		
Peer review of articles for scientific journals	2022	1,0

[1] 1ECTS (European Credit Transfer System) is equal to a workload of 28 hours
[2] Due to the COVID pandemic I was not able to attend the international conferences planned

ABOUT THE AUTHOR

Carolina Silva was born on October 18th 1987 in Niterói, Rio de Janeiro, Brazil. As a little child she desired to become a dentist as her aunts. During her secondary school, she discovered a passion for the human body and a mission for helping people. In 2008, she started her medical education at Federal University of the State of Rio de Janeiro. During her clinical internships she developed a specific interest in the field of Pediatrics. After obtaining her medical degree in 2014, she started her residency in pediatrics at Antonio Pedro University Hospital in Niterói. In 2016, she became a pediatrician and continued her education with a Neonatology fellowship. One year later, she moved to the Netherlands with her husband and shortly after started an internship in the Neonatology Intensive Care Unit at Sophia Children Hospital under the supervision of Prof.dr. I.K.M. Reiss and dr. D.W.E. Roofthooft. In 2018, after 3,5 years of clinical work, she became a PhD candidate at the Generation R Study Group at the Erasmus MC Rotterdam under the supervision of Prof. dr. Vincent Jaddoe, Prof.dr. H. El Marroun and dr. S. Santos. During her project, she coordinated the children carotid ultrasound measurement at 10 years and obtained her Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES) in 2021. She will continue her journey, combining her medical and scientific background, outside academia.

WORDS OF GRATITUDE

First of all, thanks to God, who gave me the strength to never give up, the necessary wisdom and made the impossible possible. It was never coincidence, it was always You.

Second, this thesis would not have been possible without the support of my supervisors, co-authors, colleagues, friends and family. I would like to take this opportunity to express my sincere gratitude and appreciation to all of you. I am also grateful to the people who founded the cohort, all the staff, children and their parents for the commitment and dedication to participate in the Generation R and making our research possible.

Prof.dr. Jaddoe

To my promotor. Dear Vincent, I would like to thank you for giving me the opportunity to work at the Generation R Study Group. Even though you did not know me well, you invested on me and gave me the chance to build a research career. I am truly grateful for every challenge that I faced and for everything that I learned since I am in your research group. That was a life changing experience for me.

Prof.dr. El Marroun

To my promotor. Dear Hanan, I would like to thank you for allowing me to grow as a researcher and for being always so nice and supportive during all the different moments that I experienced in the past 4 years. I was very lucky to have you as supervisor. Also, thank you for the constant encouragement and optimism regarding my PhD and for always finding time to help and teach me. Beyond work, I am grateful for the opportunity to know you in a more personal level and for all our coffee breaks and talks.

Dr. Santos

To my co-promotor. Dear Susana, you have been for me much more than a supervisor, you became a friend and have been part of my family in the Netherlands. I would like to thank you for making so palpable every day your faith on me, on my work. I am grateful for all your time, work and expertise since my first project. You have been by my side, helping and giving all the support that I needed during my PhD trajectory.

Committee

Dear reading committee members, Prof.dr. Reiss, Prof.dr. M.H.J. Hillegers, and Prof.dr. T.J. Roseboom, thank you for agreeing to read and evaluate my thesis. I also kindly thank Dr. R. Gaillard, Dr. M. Kavousi and Dr. T. Vrijkotte for agreeing to be the members of the plenary committee during my PhD defense. It is a great honor to have all of you as members of my doctoral committee.

Co-authors

Dear co-authors, thank you for your collaboration and relevant scientific input in the manuscripts of this thesis. Specifically, I would like to thank Flo for being so nice since the first time we met, for our collaboration in two projects and for all the enjoyable and funny coffee talks. Ryan, thank you for your help and patience. Without your support my IMT task and the brain imaging projects would not have been possible. Janine, although you were not directly involved as my supervisor, thank you for welcoming me in my first day and for being so helpful and nice.

Paranymphs

Dear Bruna and Caro, thank you for being such great friends and also the greatest paranymphs ever. Bruna, I will always be grateful to my IMT task since it was the reason that we shortly shared the same office and it was the topic of our first talk. Thanks for all coffee breaks, nice talks, jokes and fun that ended up in a nice and certainty long friendship. Caro, I am grateful for your Brazilian connections, specially Raquel, that introduced us in my first months at Generation R. I am very happy we met and I am so glad to see Lucas and Arturo growing together. Thank you for knowing me so well and having always the right words to say.

NICU colleagues

All this PhD adventure, would not have happened if Dr. Daniella Roofthooft and Prof.dr. Irwin Reiss, would not have accepted my internship in the Neonatology Intensive Care Unit at Sophia Children Hospital and established the contact with Vincent. I am grateful we met and had the opportunity to shortly work together. Dear Lisa, thank you for your warm welcome and for being so friendly and nice.

Generation R colleagues

During these PhD years, I have met nice colleagues who made this period enjoyable and unforgettable. The list of names is huge to be able to mention all, but you know who you are. Thank you for keeping up a friendly atmosphere out of the work environment. Also, thanks for the nice talks about work and non-work related topics, and for the fun during coffee or lunch breaks. Dear Raquel, Paula, Nathalie, Monica, Laura, Lea, Yllza, and Sunayna, I am grateful we met in the beginning of my journey at the Generation R and had the opportunity to know each other beyond work. Thank you for your friendship and for all funny talks, and coffee breaks. Dear Annemiek, Chalana, Patrícia, Kim and Hugo, apart from your support on classes and research during the NIHES courses, thank you for making this time so enjoyable. Dear Irene, thank you for being so open and nice since the first time we met. It was a great pleasure to trained you on the IMT measurements. Dear ScanLab team, even though I was almost finishing my PhD, I am grateful to have had the chance to meet all of you.

Rotterdam Family

Dear Petroleros, you received Mario so well that made us believe that would be possible to live in the Netherlands. Thank you for your warm welcome and friendship. Dear friends from Hope Church, you received us as family since we arrived and you were always there for us when we needed. I am grateful to have your support and friendship. Queridos Carol, Breno, Vava, Tati, Paulo, Bentinho, Fernanda, Diogo, Mabel, Kauer, Stellinha, Lígia, Ayres, Pedro, Lucas, Issa, Fabi e Sosô, não tenho palavras para agradecer a amizade de vocês. Vocês se tornaram nossa família aqui na Holanda e fazem nossos dias muitos mais leves e felizes. Queridos amigos da Credo, sinto diariamente o cuidado e amor de Deus com a minha família através da vida de vocês. Sou muito grata pelo nosso encontro. Obrigada por todo carinho, apoio e conversas, vocês já ocupam um lugar muito especial no meu coração.

Amigos

Queridas Ana, Dani, Lili, Livia, Pih e Rê, certamente vocês estiveram em todos os momentos importantes da minha vida. Não tenho como agradecer a amizade e o apoio de vocês durante tantos anos. Como eu queria vocês aqui comigo, vocês fazem parte da minha saudade diária. As de sempre para sempre. Queridos Vivi, Andrews, Liana, Everton, Rapha, Dudu, Isa, Ludi, Emilly e Brenda, como sou grata por compartilhar minha vida com vocês e ter a sorte de ter pessoas tão especiais como amigas. Mesmo que fiquemos muito tempo sem nos falar ou que a distância e o fuso atrapalhem, quando estamos juntos nada muda, parece que nos vimos ontem. Obrigada pelo carinho, conversas, risadas e pela certeza que posso contar com vocês. Queridas Benhês, como sou grata pela UNIRIO ter me dado vocês. Que sorte a minha ter vocês durante os longos anos de faculdade. Vocês tornaram o estudo, as provas e apresentações de grupo muito mais divertidas. E que time nós formamos, heim? Sinto muita falta do nosso convívio diário. Mas sei que mesmo nossas vidas tendo tomado caminhos diferentes, a amizade e o carinho permanecem os mesmos. Queridas Ritinha, Carol, Bia e Jubs, não sei o que seria de mim sem vocês durante a residência. Obrigada pela amizade, carinho, e por trazerem leveza à rotina tão pesada do hospital. Queridas amigas do CHN, em especial minhas duplas do coração (Assunção e Telmix), obrigada por serem as melhores companhias de plantão, por todo apoio, e por me ensinarem tanto. Queridos da UTI Neo da UFF, mesmo nosso convívio tendo sido por menos tempo do que o esperado guardo com muito carinho as lembranças do meu período com vocês. Obrigada pela amizade, paciência, e inúmeros ensinamentos.

Família

Queridos pais, irmão, avós, sogros, cunhada, tios e primos, eu não estaria aqui hoje sem o amor e apoio incondicional de vocês. Vocês sempre acreditaram em mim, mesmo quando nem eu mesma acreditava. Sou muito grata a Deus pela vida de cada um de vocês e pelo privilégio de ter vocês comigo em todos os momentos. Um especial obrigada `a minha mãe

e irmão por nunca medirem esforços para estarem comigo e fazerem eu me sentir a pessoa mais especial e amada do mundo. Querido Marinho, meu melhor amigo e companheiro de vida, eu não tenho como descrever tudo o que você representa para mim. Você já era o ser humano mais incrível que eu conhecia, mas após se tornar pai você conseguiu se superar e me surpreender ainda mais. Só tenho a agradecer por todo seu amor, carinho, paciência, e apoio em todos os momentos da minha vida. Eu não conseguiria nada disso sem você. Obrigada por cuidar tão bem de mim, da nossa família. Querido Lucas, você chegou e virou meu mundo de pernas para o ar. Obrigada por me permitir enxergar o mundo através dos seus olhos e por trazer um novo significado na minha vida. Sou grata pelo turbilhão de sentimentos que você me permite experimentar, por me fazer descobrir o amor incondicional, e por me fazer querer sempre buscar minha melhor versão.

"It is good to have an end to journey toward; but it is the journey that matters, in the end."

Ursula K. Le Guin