Cardio-metabolic risk in children prenatally exposed to maternal psychosocial stress

van Dijk, A.E.

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The associations of prenatal maternal psychosocial stress with BMI and blood glucose metabolism in offspring at 5-6 years of age

Aimée E van Dijk, Manon van Eijsden, Karien Stronks, Reinoud JBJ Gemke & Tanja GM Vrijkotte.

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ABSTRACT

**Objective:** Does prenatal maternal psychosocial stress affect BMI and blood glucose profile in their children aged 5-6 years?

**Study design:** In the Dutch ABCD cohort, depressive symptoms, pregnancy-related anxiety, parenting daily hassles and job strain were recorded by questionnaire (gestational week 16). A cumulative stress score was also calculated. BMI (n=3,168), fasting glucose (n=1,970), C-peptide and insulin resistance (n=1,495) were assessed in children at 5-6 years.

**Results:** The single and cumulative stress scales were not associated with glucose/C-peptide/insulin resistance. Pregnancy-related anxiety and high job strain were associated with higher offspring BMI. BMI was also significantly higher in the highest cumulative stress category (all associations p=0.02).

**Conclusion:** Pregnancy-related anxiety, job strain and prenatal cumulation of stress were associated with higher BMI in offspring at age 5-6, but not with parameters of fasting glucose metabolism. Differences might still emerge later in life or in response to a metabolic challenge.
The global epidemic increase in obesity and type 2 diabetes evidently reflects changes in lifestyle. As in the case of lifestyle factors, markers of early life experiences, including fetal growth, may account for a similar proportion of the incidence of the metabolic syndrome.

Maternal psychosocial stress is one factor that contributes to the fetus’ early life experience. It has been shown to predict low birth weight and preterm delivery in humans, although not all studies have replicated these findings. In turn, low birth weight for gestational age and preterm delivery have been associated with adiposity, altered glucose tolerance, and type 2 diabetes in later life. Tse et al. reported an association between cumulative stress during pregnancy and corticotrophin-releasing hormone (CRH) concentrations, in separate analyses of Blacks and Hispanics. These findings indicate that the accumulation of stress heightens allostatic load, as posed by McEwan and Stellar in 1993.

Evidence linking prenatal stress to offspring glucose metabolism is mainly available from animal studies administering corticosteroids. For instance, from studies in rats we know that prenatal exposure to excess maternal glucocorticoids causes hyperglycemia and glucose intolerance. Human studies appear to be scarce. A 30-year follow-up of an RCT on betamethasone administration provided evidence of fetal programming of glucose metabolism (insulin resistance) in adult life. However, glucocorticoid treatment usually takes place when preterm birth is expected, adding bias because of the complications of premature birth. Another prenatal form of stress, psychosocial complaints, are also of importance because they are highly prevalent in otherwise normal pregnancies. Psychosocial stress is accompanied by increases in naturally-occurring stress hormones.

A few human studies have reported associations between prenatal depression or bereavement and adiposity in children. Entringer et al also focused on prenatal psychosocial stress exposure, and observed higher insulin resistance in prenatally stressed young adults, in response to an oral glucose tolerance test. To the best of our knowledge, no (human) studies to date have examined whether the putative relationship between prenatal psychosocial stress and changes in glucose metabolism in the offspring already occurs before adulthood. Therefore, the aim of this study is to examine maternal psychosocial stress during pregnancy as a potential determinant of the offspring’s BMI and glucose metabolism at 5-6 years of age. We will also explore possible effect modification by sex. We hypothesize that the presence of psychosocial stressors is associated with higher BMI, fasting glucose and C-peptide, and more glucose intolerance.

**Materials and Methods**

The present study is part of the Amsterdam Born Children and their Development (ABCD) study, a longitudinal birth cohort study. Approval was obtained from the medical ethical committees of all participating hospitals and the Registration Committee of Amsterdam. All participants provided written informed consent for themselves and their children.

**Study population**

In 2003-2004, 12,373 Amsterdam women who first attended antenatal care in Amsterdam were approached to participate. Of these women, 8,266 (67%) returned the pregnancy questionnaire, which included multiple psychosocial stress instruments (phase 1). For singleton live births, 6,735 mothers provided permission for follow-up. When the children turned five, the addresses of 6,161 mothers were retrieved from the Youth Health Care registry; attrition in this follow-up number was largely due to untraceable changes in address or migration. The
mothers received a questionnaire, including an informed consent sheet for a health check of their child. The health check itself consisted of various health measurements in 3,321 children (2008-2010) 30.

The current study population (figure 1) included only mother-child pairs in which: the mother had completed the psychosocial questions during pregnancy (mean gestational age 16 weeks, interquartile range 13-19); the child participated in the age five health check, including BMI assessment; and data on all covariates was available. 3,168 mother-child pairs were included in the current study’s analyses regarding offspring BMI. The population for blood glucose analyses was further reduced because not all participating parents consented to the finger prick test for their children, and the child should have been fasting (n=1,970). For part of the population the amount of capillary blood obtained was too small to estimate C-peptide concentrations (C-peptide n=1,495).

Figure 1 Procedure of the ABCD study cohort and inclusion in the current analyses
Independent variables: maternal prenatal psychosocial stress

State anxiety was assessed using the Dutch version of the State-Trait Anxiety Inventory (STAI) 31. The 20 items regarding state anxiety (transient or temporarily experienced anxiety) were included in our questionnaire, with each item scored on a 4-point scale.

Depressive symptoms were assessed using the validated Dutch version of the 20-item Center for Epidemiological Studies Depression Scale (CES-D) 33,34. This scale evaluates the frequency of depressive symptoms experienced during the preceding week. Each item was scored on a four-point scale.

Pregnancy-related anxiety was assessed using an abbreviated 10-item version of the Pregnancy Related Anxieties Questionnaire (PRAQ) 35. Each item was scored on a four-point scale. In the current study, not the three underlying constructs, but the overall score was used.

To assess parenting stress, a Dutch adaptation of the 20-item Parenting Daily Hassles (PDH) scale was used 38. The parents rated the occurrence of typical everyday events in parenting and parent-child interactions on a four-point scale.

To assess job strain, a Dutch version of the Job Content Questionnaire was used 39. This questionnaire consists of two subscales: job demands (25 four-point scale items on work pace, mental workload and physical workload) and job control (11 items). The total score of the job demands scale was dichotomized using the 80th percentile as the cut-off and the job control scale was dichotomized using the 20th percentile as the cut-off to create four (2×2) categories of job strain. Jobs that are high in demands and low in control are considered most stressful (high job strain).

A cumulative total stress score was calculated by ascribing points to the number of times a mother scored above the 80th percentile for three of the above mentioned stress scales (depressive symptoms, pregnancy-related anxiety and parenting stress). A fourth point was added if the mother scored ‘high’ on the job strain scale. This approach resulted in a sum score between 0 and 4, which was divided into: No stress (0 stressors), 1 stressor, 2 stressors, and 3-4 stressors. State anxiety was not included in this score because of its high correlation with depressive symptoms (correlation coefficient 0.9).

Dependent variables

As a part of the age five health check, fasting capillary blood was collected in the morning. We used an ambulatory collection kit (Demecal kit: LabAnywhere, Haarlem, The Netherlands) 41 to determine plasma glucose (mmol/l) and C-peptide (nmol/l). The C-peptide variable was left-censored: 49% of the assessed C-peptide concentrations fell below the detection limit of 0.34 nmol/l. Therefore, associations with C-peptide were explored using survival analysis. In order to quantify insulin resistance, the latest homeostatic model assessment (HOMA2-IR) was used (Diabetes Trials Unit, University of Oxford; http://www.dtu.ox.ac.uk/homa). We used the children’s sex, age and BMI to predict C-peptide concentrations for the missing cases with survival analysis in R (‘survreg’), applying the log-logistic distribution because it was the best fitting, based on log-likelihood (R 2.13.0, R Foundation for Statistical Computing, Vienna, Austria).

Covariates

The origins and definitions of most of the covariates have been described previously 30. The included covariates were: maternal age, ethnicity, pre-pregnancy BMI, educational level, smoking and alcohol consumption in pregnancy, hypertension (no/pre-existent/pregnancy-
induced), parity, gestational duration, standardized birth weight, sex and BMI of the child.

Statistics
Descriptive statistics were run in SPSS (SPSS, Chicago, Illinois, USA). Associations of stress scales with BMI, glucose and HOMA2-IR were explored using ordinary least squares linear regression analysis (‘ols’) in R (R Foundation for Statistical Computing). Associations of stress scales with C-peptide were explored using survival analysis (‘survreg’), applying a log-normal distribution. All analyses were standardized for sex and age of the child by default (model 1). All potential confounders were determined a priori and added simultaneously in model 2 (=model 1+maternal age, ethnicity, pre-pregnancy BMI, educational level, parity, hypertension, smoking, alcohol consumption, standardized birth weight, gestational duration and BMI of the child -if applicable). Associations are also checked for linearity using restricted cubic splines. Effect-modification by sex was tested by adding an interaction term to model 2. Betas of depressive symptoms, state anxiety, pregnancy-related anxiety and parenting daily hassles were multiplied by ten to obtain a more comprehensible interpretation of the data.

RESULTS
The characteristics of the mothers and children are presented in Table 1. BMI of the child was positively correlated with glucose, C-peptide and HOMA2-IR (all p<0.01; data not shown).

Table 1 Maternal and offspring characteristics (n=3,168)

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<th>Interquartile range</th>
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<table>
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<th>Lower</th>
<th>Upper</th>
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<td>25.8</td>
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<td>Dutch ethnicity (% yes)</td>
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<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
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<td>Primiparous (% yes)</td>
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<td>Hypertension: Pre-existing hypertension (%yes)</td>
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<td>Pregnancy hypertension (%yes)</td>
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</tr>
<tr>
<td>State anxiety</td>
<td>37</td>
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<td>Pregnancy-related anxiety (total score)</td>
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<td>5</td>
<td>16</td>
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<td>(n=1,381) Parenting daily hassles</td>
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<td>Moderate job strain (%yes)</td>
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<td>High job strain (%yes)</td>
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<td>Cumulative stress score: No stress (%yes)</td>
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<td>1 Stressor (%yes)</td>
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<td>2 Stressors (%yes)</td>
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<tr>
<td>3-4 Stressors (%yes)</td>
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<tr>
<td>Child – At birth Sex (% boys)</td>
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<td>Gestational duration (weeks)</td>
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<td>Premature (&lt;37 weeks) (% yes)</td>
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<td>Birthweight (g)</td>
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<td>552</td>
<td>2800</td>
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<tr>
<td>Child – At age 5 measurement Age (y)</td>
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<td>5.0</td>
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<td>BMI (kg/m²)</td>
<td>15.5</td>
<td>1.5</td>
<td>13.8</td>
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<tr>
<td>(n=1,970) Glucose (mmol/l)</td>
<td>4.6</td>
<td>0.5</td>
<td>4.0</td>
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<tr>
<td>(n=1,495) C-peptide (nmol/l)</td>
<td>0.35</td>
<td>0.11</td>
<td>0.25</td>
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<tr>
<td>(n=1,495) HOMA2 insulin resistance</td>
<td>0.74</td>
<td>0.25</td>
<td>0.54</td>
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</table>

The betas and confidence intervals of the associations between the stress scales and outcome measures are presented in Table 2. Depressive symptoms, state anxiety, pregnancy-related anxiety, parenting daily hassles, job strain and cumulative stress were not associated with glucose, C-peptide or insulin resistance, both before (model 1) and after full adjustment for confounders (model 2).

After minimal adjustment (model 1), associations with offspring BMI were observed for prenatal depressive symptoms (β×10=0.119 kg/m², 95%CI [0.055;0.183]; p<0.01), state anxiety (β×10=0.098 kg/m², 95%CI [0.045;0.150]; p<0.01), pregnancy-related anxiety (β×10=0.240 kg/m², 95%CI [0.128;0.352], p<0.01), job strain and cumulative stress (both p for trend<0.01).

After full adjustment, statistically significant but small associations remained between pregnancy-related anxiety and offspring BMI (β×10=0.134 kg/m², 95%CI [0.020;0.247]; p=0.02) and between job strain and offspring BMI (high job strain β=0.216 kg/m², 95%CI[0.003;0.428]; p=0.0047, p for trend<0.01). Overall, increasing cumulative stress was borderline associated with BMI (p for trend=0.06); however, BMI was 0.373 kg/m² (95%CI [0.058;0.688]; p=0.02) higher in the highest cumulative stress category (3-4 stressors) compared to the low stress category (no stressors).
Table 2 Associations of maternal prenatal stressors with BMI and blood glucose metabolism measures in the resulting children at 5-6 years of age

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
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<td></td>
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<td>C-peptide</td>
<td></td>
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<tr>
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<td>(n=3,168)</td>
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<td>(n=1,495)</td>
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<td>Depressive symptoms</td>
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<tr>
<td>(β×10)</td>
<td>0.119</td>
<td>0.055-0.183</td>
<td>0.000</td>
<td>-0.065-0.064</td>
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<td>State anxiety (β×10)</td>
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<td>0.045-0.150</td>
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<tr>
<td>Parenting daily</td>
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<td>hassles (β×10)</td>
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<td>0.098-0.098</td>
<td>-0.079</td>
<td>-0.183-0.025</td>
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<td>(β×10)</td>
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<td>-0.032-0.057</td>
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Notes:

a. Model 1: Adjusted for age, sex, and age at delivery.
b. Model 2: Additional adjustment for maternal education, income, smoking, alcohol use, and parity.
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<tr>
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<th>HOMA2 insulin resistance (n=1,495)</th>
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<tr>
<td>State anxiety (β×10)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Pregnancy-related anxiety (β×10)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Parenting daily hassles (β×10)&lt;sup&lt;d&lt;/sup&gt;</td>
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<tr>
<td>High job strain</td>
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<sup>a</sup> Sex and age of the child at time of assessment were added to model 1 as covariates.
<sup>b</sup> Additional covariates added to model 2 are: maternal age, ethnicity, pre-pregnancy BMI, educational level, parity, hypertension, smoking, alcohol consumption, standardized birth weight, gestational duration (and BMI of the child, if applicable).
<sup>c</sup> Analyzed in subgroup of women already parenting (n=1,381)
<sup>d</sup> Analyzed in subgroup of women already parenting (n=877)
<sup>e</sup> Analyzed in subgroup of women already parenting (n=685)

<sup>f</sup> Each 1-unit increase in depressive symptoms/state anxiety/pregnancy-related anxiety/parenting daily hassles increases the mean BMI/glucose/C-peptide/insulin resistance with β mmHg
<sup>g</sup> The betas per job strain-category indicate the mean difference in mmHg as compared to the low job strain-category
<sup>h</sup> The betas per cumulative stress score-category indicate the mean difference in mmHg as compared to the no stress-category

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Table 2: Associations of maternal prenatal stressors with BMI and blood glucose metabolism

- **Model 1**: Analyzed in subgroup of women already parenting (n=685)
- **Model 2**: Analyzed in subgroup of women already parenting (n=877)
Gestational duration was not associated with BMI of the child, and therefore not a mediating variable in the associations between prenatal psychosocial stressors and the child’s BMI. Standardized birthweight was positively associated with BMI (p<0.01), but it was also not a mediating variable in the associations between prenatal psychosocial stressors and the child’s BMI.

The interaction term sex*independent variable was not statistically significant in any of the equations (all p≥0.05). Therefore, there was no indication that a potential association between maternal stress and any of the outcomes would differ between boys and girls.

**COMMENT**

Pregnancy-related anxiety, job strain and the cumulation of stress during pregnancy were associated with small increases in BMI in offspring at 5-6 years of age. Psychosocial stress was not associated with (unfavorable) parameters of fasting glucose metabolism.

**Comparison to existing literature**

In previous work, we have already studied the association between job strain during pregnancy and body composition in the child at 5-6 years of age. We reported that there was no association 42, which is not in concordance with the findings presented here. This difference can be attributed to the addition of 229 mother-child pairs in the present study which increased the power to detect significant differences while the effect size remained the same. Our current finding regarding childhood BMI is in agreement with a comparable, Danish study on the relation between prenatal stress exposure (maternal bereavement) and the risk of childhood BMI/overweight. Although Li et al observed higher BMI in the exposed children, the differences did not become significant until the children were 10 years old.27 Also, maternal bereavement might exert a more pronounced effect than the measures of psychosocial stress used in the present study. Ertel et al 26 observed increased central adiposity and smaller size in 3-year old children from mothers who reported high depressive symptoms prepartum. We did not replicate this finding with depressive symptoms, but we did observe higher BMI in children born from mothers who reported 3-4 psychosocial stressors during pregnancy.

Previous research has linked low standardized birth weight for gestational age with an increased risk of obesity and adiposity later in life 10. In the present study, when exploring standardized birthweight as a potential mediator, this pattern was, however, not observed: Higher standardized birthweight was associated with higher childhood BMI, also when nonlinear modeling was allowed for.

Our null finding regarding glucose metabolism is not in agreement with findings from the single most comparable human study, by Entringer et al 28. There are, however, four major differences that could explain this inconsistency. First, Entringer et al assessed glucose, insulin and C-peptide in response to an oral glucose tolerance test (a metabolic challenge), as did previous animal studies on programming by glucocorticoids 18,20. Second, the only significant difference in baseline concentrations observed by Entringer et al was that fasting insulin concentrations were 58% higher in the exposed group, an outcome measure we did not have. In agreement with the present study, Entringer et al did not observe any other significant differences in baseline concentrations of glucose or C-peptide. Third, they used major stressful life events as a determinant, which, like maternal bereavement, might exert a more pronounced effect than the measures of psychosocial stress used in the present study. Fourth, the offspring that Entringer et al reported on were young adults, not children.
The offspring in the present study are still relatively young. Most previous studies measured outcomes in adult human, rat, and sheep offspring. Possibly, the programming effects are not yet visible in baseline concentrations. We speculate that a glucose tolerance test, and/or follow-up throughout adulthood, might yield different results. The possibility of an age-effect is supported by a striking difference between two rat studies. D’Mello et al. and Lesage et al. examined the effects of prenatal maternal stress in offspring at 120 days (young adult) and 24 months (adult), respectively; the former did not observe any associations with measures of glucose metabolism, while the latter reported hyperglycemia and glucose intolerance. In contrast, Moss et al. did observe increased insulin responses in 6-month-old lambs, which are relatively young. However, it must be borne in mind that this study concerned glucocorticoid administration, which exerts circulating levels that are much higher than the naturally occurring fluctuations in glucocorticoids that occur as a result of psychosocial stress. Hence, the programming effect may have been larger and thus easier to detect in those studies.

Potential mechanisms
The understanding of the pathway through which prenatal stress exerts its putative effect on glucose metabolism is limited, but several mechanisms have been suggested. It likely involves fetal exposure to glucocorticoids. Stress-induced alterations of the maternal HPA axis lead to hyper-secretion of the glucocorticoid cortisol, which partly reaches the developing fetus. In rats prenatally exposed to excess glucocorticoids, Nyirenda et al. uncovered increased hepatic expression of glucocorticoid receptor (GR) mRNA and phosphoenolpyruvate (PEPCK) mRNA, which may promote glucose intolerance by increasing gluconeogenesis. Alternative mechanisms involve inflammation markers: maternal psychosocial factors can contribute to increased inflammation during pregnancy, which has been associated with preterm birth, subsequently leading to low birth weight. The influence of maternal inflammation-linked preterm birth on the development of adult cardiovascular disease has not been extensively investigated.

Strengths and limitations
The current study was conducted in a large, multi-ethnic cohort. However, as in most cohort studies, selective loss to follow-up was present. Stress was more prevalent in the ABCD cohort as a whole than it is in the current subgroup, which is now a slightly healthier reflection of the population (i.e. higher educational level, lower BMI). Thus, the proportion of pregnant women in the highest stress category might be higher at the populational level. This underrepresentation may have resulted in an underestimation of the actual associations. However, most associations in this study are far from statistically significant: even in a population with higher stress levels, those associations are not very likely to become significant.

Methods used to assess maternal stress vary greatly among studies in the field of developmental origins of health and disease. We chose to use a score of multiple validated psychosocial stress constructs to identify women subjected to high levels of stress occurring in a normal pregnant woman’s day-to-day life, and therefore with a putatively high level of generalizability. The assessment of stress took place in the first week of the second trimester, thus assessing experienced stress in the preceding first trimester-weeks. The near the end of the first trimester is often considered the trimester with the highest fetal vulnerability because of the development of critical, basal systems, including the formation of beta cells as true
endocrine cells by the end of the first trimester of human pregnancy. Unfortunately, we did not have multiple measurements throughout pregnancy to test this hypothesis.

Predicting censored C-peptide concentrations in a substantial proportion of the population is a major limitation of the current study, which may have induced error. Also, the smaller number of available data for metabolic markers might be responsible for the null findings. However, if either were the case, we would have expected to see (at minimum) a subtle trend in the hypothesized direction regarding either C-peptide or glucose.

We did not include the child’s energy intake and physical activity in our models, because the fetal programming effect might be mediated by high energy intake of the offspring: high maternal cortisol levels may be associated with altered adiponectin metabolism in the offspring, a hormone that mediates energy consumption. Moreover, the offspring of stressed mothers may have a preference for high-energy foods or altered appetitive traits, as suggested by the results of animal studies. Therefore, adjusting analyses for post-natal feeding and eating behavior could be over-adjusting.

Moreover, despite the adjustment for many potential confounders, there is always a chance of residual confounding being present, especially when studying an outcome variable that might be influenced by many demographic and lifestyle factors, like BMI. One source of residual confounding may be postnatal stress, a measure we were not able to adjust for in the current study. Also, the levels of significance need to be interpreted with caution, because multiple comparisons are made.

In sum, the current study provides additional evidence that maternal psychosocial stress during pregnancy is associated with increased BMI in the resulting offspring. However, psychosocial stress was not associated with (unfavorable) parameters of fasting glucose metabolism. It is possible that this fetal programming effect does not exist because maternal psychosocial stress during pregnancy does not exert sufficient strain on offspring. Alternatively, differences might still occur later in life or in response to a metabolic challenge.
REFERENCES


