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

van Dijk, A.E.

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The relation of maternal job strain and cortisol levels during early pregnancy with body composition later in the 5-year-old child

ABSTRACT

Background: Prenatal exposure to maternal stress may program the fetal HPA axis, potentially leading to altered metabolism in later life, associated with adiposity and diabetes.

Aims: This association is little studied in humans, and thus we explore whether high maternal job strain during early pregnancy, as well as maternal cortisol levels are associated with increased body mass index (BMI), central adiposity or body fat mass in the offspring at age five. Additionally, we explore whether these associations are modified by gender or mediated by gestational duration and fetal growth restriction.

Study design: 2,939 Pregnant women (ABCD cohort study) completed a questionnaire around gestational week 16 including the Job Content Questionnaire, assessing job strain. Serum total cortisol was assessed in a subsample (n=1,320). Gestational age at birth (≥ 37 weeks), standardized birth weight and information on many covariates were available. At the age five health check, height, weight (BMI, kg/m²), waist circumference (waist-to-height ratio, WHtR) and Fat Mass Index (FMI, kg/m²) were assessed.

Results: Job strain was not associated with higher BMI, WHtR or FMI. Higher maternal cortisol was independently associated with marginally higher FMI in girls, but marginally lower FMI in boys (β 0.09 and β -0.10 per 100 unit increase in serum cortisol, respectively. p<0.01). This association was not mediated by gestational duration or fetal growth restriction.

Conclusions: Results show that prenatal maternal job strain and cortisol may not program obesity and adiposity in the next generation in humans, but gender differences should always be considered.
The prevalence of overweight and obesity in children has tripled over the past decades. This increase may be largely attributed to decreased physical activity and increased caloric intake. However, accumulating evidence from observational as well as animal studies shows that other factors, acting in early life, are probably also involved.

According to the ‘fetal programming hypothesis’, prenatal exposure to suboptimal intrauterine conditions could predispose the individual to chronic disease at adult age. Such conditions could be exposure to maternal malnutrition, but also exposure to psychosocial stress. Stress can be defined in numerous ways, ranging from self-reported psychosocial complaints, e.g. experienced life events, anxiety, depression and job strain, to physiological indicators, such as glucocorticoids. Despite the increasing body of evidence from animal studies, so far there have only been two known studies in humans relating prenatal maternal psychosocial stress to body composition in the offspring during childhood. The study by Li et al. however only used BMI as a measure of body composition, and in the study by Ertel et al., body composition was already measured at age three.

The present study therefore aims to examine the relation of prenatal stress exposure with the child’s body composition. We chose to study job strain as the psychosocial stressor as it is highly prevalent in women of reproductive age, but receives less attention than, for example, depression or anxiety. Moreover, job strain can be a chronic stressor and related to altered HPA-axis activity, leading to higher mean cortisol or altered cortisol awakening responses; it therefore has the potential to program the fetus’ HPA-axis. As a physiological measure of stress, we also explore whether high maternal cortisol status during early pregnancy is associated with adiposity measures in the offspring at age five.

Lastly we will examine sex-specific effects, given the accumulating evidence of sex-specific effects in fetal programming, with males generally found to be more sensitive to stress in utero, and the mediating role of gestational duration and birth weight, given the evidence that these factors may mediate the association between high maternal cortisol and adverse offspring outcomes.

We hypothesize that high prepartum stress is associated with offspring (central) adiposity, with increased sensitivity to this effect in boys and mediation by shorter gestational duration and lower birth weight.

**METHODS**

**Study population**

The current study is part of a prospective birth cohort, the Amsterdam Born Children and their Development (ABCD) study (http://www.abcd-study.nl). Figure 1 visualizes the procedure of inclusion in the present study.
All pregnant women from Amsterdam visiting an obstetric care provider between January 2003 and March 2004 were asked to fill out a questionnaire. Of 12,373 women, 8,266 (67%) completed the questionnaire (at a median gestational age of 16 weeks, IQR 12-20) and a focus group of 4,389 (53%) participants also donated a blood sample for biomarker analysis, including serum cortisol. A total of 6,735 (81%) women with live-born singletons gave permission for follow-up.

In 2008, when the children reached the age of five years, the mothers for whom an address could be retrieved (N=6,161) received a questionnaire, including an informed consent sheet for the age five health check, which was returned by 73%. The health checks (N=3,287) took place between September 2008 and December 2010.

The current study population included only mother-child pairs with available job strain and body composition data. Births had to be at term (gestational duration ≥ 37 weeks) and information on the covariates had to be available. In the analyses regarding cortisol, mothers with pre-existent diabetes were excluded, as well as mothers who came in for blood sample donation after gestational week 20. The time of day of blood sampling had to be available. None of the mothers in the remaining sample were using steroid medication.
Independent variable: job strain
To assess work stress (or job strain), a Dutch version of the Job Content Questionnaire (JCQ) was included in the self-administered pregnancy questionnaire. It consists of 2 subscales: job demands and job control. The job demands subscale consists of 25 four-point scale items focusing on work pace, mental workload and physical workload. The job control subscale consists of 11 four-point scale items (Cronbach’s alpha 0.85 and 0.92, respectively).

In concordance with the JCQ-guidelines, we divided the job demand score into: low (<50th percentile), moderate (50th-90th percentile) and high (>90th percentile). Similarly, we divided the job control score into high (>50th percentile), moderate (10th-50th percentile), and low (<10th percentile). Next, the categorical variable of interest, job strain, could be determined. One of the four categories consisted of non-employed women (employment = having a paid job ≥ 8 hours per week during). Women in the category ‘low job strain’ had reported low job demand with moderate or high job control. ‘High job strain’, consisted of women who reported high job demand with low or moderate job control. All other women fell into the ‘moderate job strain’ category.

Dependent variables: body composition
Body composition of the children was determined at the age five health check. Height was measured to the nearest millimeter using a Leicester portable height measure (Seca, Hamburg, Germany) and weight to the nearest 100 gram using a Marsden MS-4102 weighing scale (Oxfordshire, United Kingdom). BMI (kg/m²) was calculated.

Waist circumference was measured to the nearest millimeter using a Seca measuring tape. Waist-to-height-ratio was calculated by dividing waist circumference (cm) by height (cm).

The children underwent two arm-to-leg bioelectrical impedance analysis (BIA) measurements using the Bodystat 1500 MDD (Bodystat Inc, Douglas, United Kingdom). For each measurement value, the mean was used in the calculations. Fat mass index (kg/m²) was calculated using the following equations, adapted from Kushner et al. and Lohman:

\[
\text{Total body water} = 0.59 \times \text{Height}^2/R50 + 0.065 \times \text{Weight} + 0.04
\]
\[
\text{Fat free mass} = c \times \text{Total body water}
\]
\[
\text{FMI} = (\text{Weight} - \text{Fat free mass}) / \text{Height}^2
\]

in which R50 is resistance at 50 kHz and c (hydration constant) is 1.30 (100/77) for boys and 1.28 (100/78) for girls.

Potential mediators and confounders
Gestational age at birth, birth weight and gender were available from Youth Health Care Registration. Birthweight was standardized for gender, gestational duration and parity (reference values from Dutch Perinatal Registration; PRN; www.perinatreg.nl). This standardized measure is considered a proxy for fetal growth restriction. When adding standardized birth weight (or fetal growth restriction) to a model, a linear and a quadratic term were added simultaneously to test for possible non-linear effects.

Maternal characteristics considered potential confounders were age, pre-pregnancy BMI (kg/m²), educational level (years of education after primary school, as a measure of socioeconomic status), parity (primipara: yes/no), ethnicity (maternal country of birth; definition by the Dutch Central Bureau of Statistics; CBS), smoking (y/n) and alcohol consumption during pregnancy (y/n), all available from the pregnancy questionnaire. Hypertension (no/pre-exis-
tent/pregnancy-induced) was available by combining data from the questionnaire and Dutch Perinatal Registration. The categories were classified in accordance with the guidelines of the International Society for the Study of Hypertension in Pregnancy (www.isshp.com). The duration of exclusive breastfeeding (<1 month, 1-4 months, >4 months) was an additional potential confounder, available from Youth Health Care Registration. The number of hours worked per week was divided into categories, following conventional Dutch classifications (<24 hours; 24-31 hours and >=32 hours per week).

Maternal cortisol status
From our study group, 1,320 women also participated in the biomarker focus group. The analyses regarding cortisol status were run on this smaller subgroup. Blood collection in the pregnant women took place at their first prenatal visit, at various gestational ages (median gestational age 13 weeks; IQR 11-15) and times of the day. Therefore, serum cortisol concentrations to be used in the analyses were standardized by regressing time of day (categorical scale) and gestational age at blood sampling (continuous scale) to cortisol values (continuous scale). For each participant, we then calculated the estimated cortisol value at the median gestational age of assessment and at time of day between 8:00-9:00 a.m., because of cortisol’s diurnal variation and its increase with pregnancy duration 37,38.

For each participant, a blood sample was sent to the Regional Laboratory of Amsterdam where 1 ml plasma and serum aliquots were prepared and stored at -80ºC until analysis. Total serum cortisol was determined by radio-immunoassay. The interassay coefficient of variation (CV) was 10.2% for low and 4.9% for high values.

Statistics
Univariate associations between descriptive variables and job strain were explored using Chi square tests and ANOVAs with post-hoc Tukey tests (SPSS 17.0, SPSS Inc., Chicago, USA). Multivariate analyses were explored using ordinary least squares linear regression analysis (R, R foundation for statistical computing, Vienna, Austria).

All potential confounders were determined a priori and added simultaneously. The gender and age of the child at measurement were included as covariates by default to all analyses involving body composition at age five. Mediation was tested using the Baron and Kenny mediation test 39 in combination with linear and logistic regression models. Effect-modification by sex was tested by adding an interaction term. Statistical significance (two-sided) was determined at α=0.05.

Results
Table 1 presents the characteristics of our study population by job strain category. This table also shows that job strain was not associated with serum cortisol (ANOVA p=0.23).
### Table 1 Maternal, Postnatal and Child Characteristics (mean (SD)) by Prenatal Job Strain Status of the Mother, ABCD Study, Amsterdam, the Netherlands, 2003-2010.

<table>
<thead>
<tr>
<th>N=2,939</th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=795, 27%</td>
<td>n=966, 33%</td>
<td>n=1,056, 36%</td>
<td>n=122, 4%</td>
</tr>
</tbody>
</table>

#### Maternal characteristics

<table>
<thead>
<tr>
<th></th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.5 (5.7)</td>
<td>32.6 (3.9)</td>
<td>32.4 (3.9)</td>
<td>31.9 (4.8)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>23.7 (4.6)</td>
<td>22.6 (3.4)</td>
<td>22.6 (3.4)</td>
<td>23.3 (3.5)</td>
</tr>
<tr>
<td>Educational level (years)</td>
<td>7.6 (4.3)</td>
<td>10.7 (2.9)</td>
<td>10.6 (3.2)</td>
<td>9.4 (3.5)</td>
</tr>
<tr>
<td>Weekly working hours</td>
<td>-</td>
<td>31.3 (7.6)</td>
<td>32.4 (8.0)</td>
<td>31.9 (7.5)</td>
</tr>
<tr>
<td>% Working ≥32 hours</td>
<td>0.0</td>
<td>60.9</td>
<td>64.0</td>
<td>61.3</td>
</tr>
<tr>
<td>% Living with partner</td>
<td>85.4</td>
<td>92.5</td>
<td>91.8</td>
<td>90.2</td>
</tr>
<tr>
<td>% Primipara</td>
<td>40.4</td>
<td>61.9</td>
<td>59.3</td>
<td>50.0</td>
</tr>
</tbody>
</table>

#### Ethnicity:

<table>
<thead>
<tr>
<th></th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Dutch</td>
<td>51.1</td>
<td>84.3</td>
<td>85.1</td>
<td>72.1</td>
</tr>
<tr>
<td>% Surinamese</td>
<td>5.5</td>
<td>2.4</td>
<td>2.8</td>
<td>3.3</td>
</tr>
<tr>
<td>% Turkish</td>
<td>7.3</td>
<td>0.6</td>
<td>0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>% Moroccan</td>
<td>13.1</td>
<td>1.6</td>
<td>1.4</td>
<td>2.5</td>
</tr>
<tr>
<td>% Other non-western country</td>
<td>16.5</td>
<td>4.8</td>
<td>3.8</td>
<td>6.6</td>
</tr>
<tr>
<td>% Other western country</td>
<td>6.5</td>
<td>6.4</td>
<td>6.4</td>
<td>8.2</td>
</tr>
<tr>
<td>% Smoking</td>
<td>10.4</td>
<td>6.6</td>
<td>8.8</td>
<td>13.9</td>
</tr>
<tr>
<td>% Consuming alcohol</td>
<td>17.4</td>
<td>31.7</td>
<td>32.1</td>
<td>18.9</td>
</tr>
</tbody>
</table>

#### Hypertension:

<table>
<thead>
<tr>
<th></th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Pre-existent hypertension</td>
<td>3.9</td>
<td>2.3</td>
<td>2.6</td>
<td>3.3</td>
</tr>
<tr>
<td>% Pregnancy hypertension</td>
<td>5.9</td>
<td>10.0</td>
<td>8.5</td>
<td>11.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cortisol (nmol/L) *</th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>490 (117)</td>
<td>474 (98)</td>
<td>479 (99)</td>
<td>477 (87)</td>
<td></td>
</tr>
</tbody>
</table>

#### Postnatal characteristics

<table>
<thead>
<tr>
<th></th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational duration (weeks)</td>
<td>40.1 (1.2)</td>
<td>40.1 (1.2)</td>
<td>40.1 (1.2)</td>
<td>40.0 (1.2)</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3498 (488)</td>
<td>3539 (483)</td>
<td>3542 (484)</td>
<td>3499 (499)</td>
</tr>
<tr>
<td>% Exclusively breast feeding &gt; 3 months</td>
<td>48.9</td>
<td>49.4</td>
<td>51.0</td>
<td>46.7</td>
</tr>
</tbody>
</table>

#### Child characteristics

<table>
<thead>
<tr>
<th></th>
<th>No job</th>
<th>Low job strain</th>
<th>Moderate job strain</th>
<th>High job strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.6 (0.4)</td>
<td>5.6 (0.4)</td>
<td>5.6 (0.3)</td>
<td>5.6 (0.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.8 (1.7)</td>
<td>15.4 (1.3)</td>
<td>15.3 (1.3)</td>
<td>15.7 (1.5)</td>
</tr>
<tr>
<td>Waist-to-height-ratio (cm/cm*100)</td>
<td>45.3 (3.2)</td>
<td>45.0 (2.8)</td>
<td>44.8 (2.8)</td>
<td>45.3 (3.2)</td>
</tr>
<tr>
<td>FMI (kg/m²)</td>
<td>4.0 (1.4)</td>
<td>3.6 (1.1)</td>
<td>3.6 (1.1)</td>
<td>3.8 (1.3)</td>
</tr>
</tbody>
</table>

* Only available from a subgroup; N=1,320. Standardized/estimated at the median gestational age of assessment (13 weeks) and at time of day between 8:00-9:00 a.m.
We compared the mother-child pairs included in the current study (N=2,939) to all other mother-child pairs that were eligible for inclusion (2,869; born at term and invited for age five health check). The included women were more employed (73.0% vs 60.7%), but high job strain was less prevalent (4.2% vs. 5.6%) (p<0.01). They were older (32.0 vs. 30.1 years old, p<0.01) and higher educated (9.8 vs. 8.4 years of education after primary school, p<0.01).

**Job strain**

The results of our regression analyses are presented in table 2. High job strain, as compared to low job strain, was not associated with BMI, WHtR or FMI after adjustment for confounders (β 0.2; 95% CI -0.1, 0.4). The no job category was borderline associated with higher BMI (β 0.1; 95% CI 0.0, 0.3) and higher FMI (β 0.1; 95% CI 0.0, 0.2), as compared to low job strain.

**Table 2** Body Mass Index, Waist-To-Height-Ratio and Fat Mass Index Differences in Children Prenatally Exposed to Different Levels of Maternal Job Strain, ABCD Study, Amsterdam, the Netherlands, 2003-2010. N=2,939

<table>
<thead>
<tr>
<th></th>
<th>Crude 1 β (95%CI)</th>
<th>P</th>
<th>Adjusted 2 β (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No job</td>
<td>0.4 (0.3, 0.6)</td>
<td>p&lt;0.01</td>
<td>0.1 (0.0, 0.3)</td>
<td>p=0.13</td>
</tr>
<tr>
<td>Low job strain</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Moderate job strain</td>
<td>0.0 (-0.1, 0.1)</td>
<td></td>
<td>0.0 (-0.1, 0.1)</td>
<td></td>
</tr>
<tr>
<td>High job strain</td>
<td>0.3 (0.0, 0.6)</td>
<td></td>
<td>0.2 (-0.1, 0.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Waist-to-height-ratio (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No job</td>
<td>0.5 (0.2, 0.8)</td>
<td>p&lt;0.01</td>
<td>0.0 (-0.3, 0.3)</td>
<td>p=0.20</td>
</tr>
<tr>
<td>Low job strain</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Moderate job strain</td>
<td>-0.2 (-0.5, 0.0)</td>
<td></td>
<td>-0.2 (-0.5, 0.0)</td>
<td></td>
</tr>
<tr>
<td>High job strain</td>
<td>0.3 (-0.2, 0.9)</td>
<td></td>
<td>0.1 (-0.4, 0.6)</td>
<td></td>
</tr>
<tr>
<td><strong>FMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No job</td>
<td>0.4 (0.3, 0.5)</td>
<td>p&lt;0.01</td>
<td>0.1 (0.0, 0.2)</td>
<td>p=0.24</td>
</tr>
<tr>
<td>Low job strain</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Moderate job strain</td>
<td>0.0 (-0.1, 0.1)</td>
<td></td>
<td>0.0 (-0.1, 0.1)</td>
<td></td>
</tr>
<tr>
<td>High job strain</td>
<td>0.2 (0.0, 0.4)</td>
<td></td>
<td>0.1 (-0.1, 0.3)</td>
<td></td>
</tr>
</tbody>
</table>

1 To the crude model, the child’s gender and age at measurement are added as covariates.
2 Covariates additionally added to the adjusted model: maternal age, pre-pregnancy BMI; educational level; smoking; alcohol consumption; hypertension; primiparity; ethnicity; exclusive breastfeeding.
Gender did not modify the effect of job strain on BMI, WHtR or FMI (interaction variable gender*job strain P=0.05; P=0.09 and P=0.30 respectively).

**Maternal cortisol**
Table 3 presents the results of regression analysis regarding maternal cortisol. Cortisol was in the crude nor the adjusted model significantly associated with BMI or WHtR. The beta’s for FMI are presented for boys and girls separately, because there was a significant interaction between maternal cortisol and gender (P<0.01). Higher maternal cortisol was independently associated with marginally higher FMI in girls, but marginally lower FMI in boys (β 0.09 and β -0.10 per 100 unit increase in serum cortisol, respectively. P<0.01).

The associations between maternal cortisol and FMI were not mediated by gestational duration, because cortisol was not associated with gestational duration (p=0.29 in boys; p=0.31 in girls). Cortisol was also not associated with birth weight (standardized for sex, parity and gestational duration), and therefore also not a mediator (p=0.81 in boys; p=0.08 in girls).

**Table 3** Changes in Children’s Body Mass Index, Waist-To-Height-Ratio and Fat Mass Index for Each 100-unit Increase in Prenatal Exposure to Maternal Serum Cortisol, ABCD Study, Amsterdam, the Netherlands, 2003-2010. N=1,320

<table>
<thead>
<tr>
<th></th>
<th>Crude 1 β (95%CI)</th>
<th>P</th>
<th>Adjusted 2 β (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m^2)</td>
<td>0.01 (-0.08, 0.06)</td>
<td>0.82</td>
<td>0.03 (-0.04, 0.10)</td>
<td>0.43</td>
</tr>
<tr>
<td>Waist-to-height-ratio (%)</td>
<td>0.06 (-0.08, 0.20)</td>
<td>0.40</td>
<td>0.12 (-0.02, 0.20)</td>
<td>0.10</td>
</tr>
<tr>
<td>FMI (kg/m2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-0.09 (-0.18, -0.01)</td>
<td>0.03</td>
<td>-0.10 (-0.18, -0.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>Girls</td>
<td>0.06 (-0.01, 0.14)</td>
<td>0.11</td>
<td>0.09 (0.01, 0.17)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1. To the crude model, the child’s gender and age at measurement are added as covariates.
2. Covariates additionally added to the adjusted model: maternal age, pre-pregnancy BMI; educational level; smoking; alcohol consumption; hypertension; primiparity; ethnicity; exclusive breastfeeding.

**DISCUSSION**
Job strain was not associated with higher BMI, WHtR or FMI. Therefore, there was also no mediation by gestational duration or fetal growth restriction. Higher maternal cortisol was independently associated with marginally higher FMI in girls, but marginally lower FMI in boys. This association was not mediated by gestational duration or fetal growth restriction.

**Mechanisms**
Theoretically, there are two plausible underlying mechanisms linking prenatal maternal stress and offspring obesity. The first one involves the hypothalamus-pituitary-adrenal (HPA) axis: stress causes high cortisol in the mother, which can directly cross the placental barrier. The fetus is protected from high maternal cortisol levels by the placental enzyme 11β-HSD2 which converts active cortisol into inactive cortisone. In the case of high maternal cortisol
and/or down regulation of 11β-HSD2, the fetus is exposed to elevated cortisol which could then lead to programmed changes in the HPA axis, which in turn is related to (abdominal) obesity and impaired glucose tolerance. An association between altered cortisol reactivity (altered HPA axis functioning) and obesity was also observed in children.

The second potential mechanism is the autonomic nervous system (ANS): stress could increase sympathetic and decrease parasympathetic nervous system activity in the mother, which in turn could program the ANS-balance in the offspring. ANS-misbalance is associated with symptoms of the metabolic syndrome, including obesity, although the underlying mechanisms remain unclear.

**Previous findings**

Previous studies examining prenatal stress focused mainly on short-term outcomes like preterm birth and birth weight (e.g. ). Studies that examine the potential association between prenatal stress and adiposity in the offspring, like the present, appear to be very scarce with most of the research being done in animal models, which may not just translate to humans. For instance, Lesage et al. observed an association between prenatal stress and consequent hyperglycemia, glucose intolerance, decreased basal leptin levels and increased food intake after a fasting period in the aged rat offspring. Mueller and Bale found an association between prenatal stress and long-term effects on body weight in mice offspring. In human children who were fetally exposed to maternal glucocorticoids, Gillman et al. observed an overall decrease in body size at the age of 3 years, but with increased adiposity. We did not replicate those findings, an inconsistency that may be due to the age differences between our studies and the patterns in BMI development in young children. In the same cohort as Gillman et al., Ertel et al. also observed increased central adiposity in children from mothers who reported high depressive symptoms prepartum. A Danish study on prenatal stress exposure related to maternal bereavement and the risk of childhood BMI/overweight was most comparable to ours. Li et al. observed higher BMI in the exposed children, but the differences did not become significant until the children were 10 years old. It could be that our children were too young to detect significant differences. Maybe the findings by Li et al. can be replicated in our cohort, but after longer follow up.

The current study takes into account both a self-reported measure of maternal psychosocial stress (job strain) as well as a physiological measure (total serum cortisol). In our sample, job strain is not associated with total serum cortisol. This lack of correlation between self-reported and physiological stress is, however, not uncommon. Data from an extensive study by Davis and Sandman indicate that, at five time points during pregnancy, maternal cortisol was not significantly associated with (pregnancy specific) anxiety or depression. A recent study by Himes et al. in second trimester pregnant women did not find a relationship between perceived stress or psychosocial status and cortisol or CRH at all. Diego et al. did report correlations between maternal cortisol and psychological stress measures, but the highest correlation coefficient was 0.4 (cortisol and depressive symptoms).

**Strengths and limitations**

In our study, cortisol was not optimally assessed, which may partly explain the aforementioned lack of correlation. For different participants, it was collected at different time points during gestation and at different times of the day. Although we did aim to standardize for gestational age and time of day at collection, it does add a certain amount of error to the re-
sulting values. A previous study among this cohort reported a possible moderation effect by time of day \(^{38}\), which was supported by other studies. Morning cortisol was associated with their outcomes, and afternoon cortisol was not \(^{54;55}\). Another issue is the assessment of total serum cortisol versus free serum cortisol levels. Although in studies regarding very specific physiological effects it would be preferable to assess free serum cortisol, in a study like the present the total cortisol may still very well be a strong proxy for free cortisol levels.

As would be expected, the non-response analysis revealed selective loss to follow-up. The lack of pronounced significant findings in our study may therefore also be due to selection bias. The borderline findings we did observe, could, despite the extensive adjustment for confounders, be the result of residual confounding or plain chance.

A strength is the first trimester-timing of stress assessment, which is often considered the trimester with the highest fetal vulnerability \(^{56}\). Additionally, Li et al. reported an indication that exposure to maternal bereavement the months just before conception may be the most susceptible period, because it may lead to excessive glucocorticoids during early pregnancy \(^{19}\). Surely, multiple measurements throughout pregnancy in our cohort would have been preferable, but not attainable.

We did not include the child’s energy intake and physical activity in our models, because the fetal programming effect is very likely mediated by high energy intake of the offspring: High maternal cortisol levels may be associated with altered adiponectin metabolism in the offspring \(^{57}\), a hormone that mediates energy consumption \(^{57;58}\). Moreover, the offspring of stressed mothers may have a preference for high-energy foods or altered appetite traits, as suggested by the results of animal studies \(^{5;6}\). Therefore, adjusting analyses for post-natal feeding and eating behavior could be over-adjusting.

**Gender differences in programming**

The currently observed adverse programming effect of cortisol in girls and favorable programming effect in boys confirms a point argued by Clifton \(^{59}\). Previous studies on gender differences have repeatedly noted higher vulnerability in male fetuses, like increased risk of distress during labor and delivery \(^{23;25;60-62}\). As potential explanatory mechanisms, 11β-HSD2 activity may be reduced in female fetuses, leaving the girls more exposed to elevated maternal cortisol, leaving them more resilient \(^{24;63;64}\). Also, sex differences in the pattern of expression of glucocorticoid and mineralocorticoid receptors during development may indicate different windows of vulnerability to prenatal glucocorticoid exposure \(^{25}\). The reason for the gender difference observed in the current study may also be sought in the evolutionary theory underlying fetal programming \(^{65}\). Being exposed to elevated stress, the fetus retains more body fat in order to be more successful at reproduction in scarce times. This is based upon the knowledge that excess adiposity in girls is hypothesized to be a causal factor for early pubertal development in girls \(^{66}\).

In conclusion, prenatal maternal job strain and cortisol may not program obesity and adiposity in the next generation in humans, but gender differences should always be considered. Fetal programming of obesity by psychosocial stress may require a longer follow-up period.
REFERENCES


