Title: Determinants of Cortisol During Pregnancy – the ABCD Cohort

Authors: Laura S. Bleker, Tessa J. Roseboom, Tanja G. Vrijkotte, Rebecca M. Reynolds, Susanne R. de Rooij

PII: S0306-4530(17)30179-8
DOI: http://dx.doi.org/doi:10.1016/j.psyneuen.2017.05.026
Reference: PNEC 3641

To appear in:

Received date: 20-2-2017
Revised date: 27-4-2017
Accepted date: 26-5-2017

Please cite this article as: Bleker, Laura S., Roseboom, Tessa J., Vrijkotte, Tanja G., Reynolds, Rebecca M., de Rooij, Susanne R., Determinants of Cortisol During Pregnancy – the ABCD Cohort.Psychoneuroendocrinology http://dx.doi.org/10.1016/j.psyneuen.2017.05.026

Laura S. Bleker

Determinants of Cortisol During Pregnancy – the ABCD Cohort

Laura S. Bleker[2], Tessa J. Roseboom[1,2], Tanja G. Vrijkotte[3], Rebecca M. Reynolds[4], and Susanne R. de Rooij[2,3]

[1]Academic Medical Centre, Department of Obstetrics and Gynecology, Amsterdam, The Netherlands

[2]Academic Medical Centre, Clinical Epidemiology, Biostatistics and Bioinformatics, Amsterdam, The Netherlands,

[3]Academic Medical Centre, Amsterdam Public Health research institute, Department of Public Health, Amsterdam, The Netherlands,

[4]University/BHF Centre for Cardiovascular Science, University of Edinburgh, United Kingdom

Address correspondence to Laura Bleker, MD, Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands. Email: l.s.bleker@amc.uva.nl

Determinants of Cortisol During Pregnancy

Highlights

- Cortisol during pregnancy is affected mainly by biological and lifestyle factors
- psychosocial stress does not seem to significantly contribute to maternal cortisol in healthy women.
- prenatal stress might program offspring outcomes through other mechanisms

Laura S. Bleker

Abstract

Background: Psychosocial stress during pregnancy has been proposed as a major contributor of glucocorticoid-mediated programming of the fetal hypothalamic-pituitary adrenal (HPA) axis, with later adverse health consequences. However, evidence linking maternal stress to maternal cortisol values during pregnancy is inconclusive. A possible explanation for this is that other maternal factors overshadow any potential effects of stress on cortisol levels. We studied a large cohort of pregnant women with extensive data on pregnancy characteristics to determine the respective contributions of biological, environmental and psychosocial stress factors to cortisol levels in pregnancy.

Methods: We used data from 3039 women from the Amsterdam Born Children and their Development-study cohort. Serum cortisol was measured in blood, collected at the first prenatal visit, at different gestational ages (median=91 days, range=40-256 days), and at various time points during the day (median=11:45h, range=08:00 – 18:30h). We assessed associations between maternal serum cortisol in pregnancy and biological factors, lifestyle factors and stress factors, including depression, anxiety, pregnancy-related anxiety, work stress, parenting stress and fatigue.

Results: In multivariable analysis, variables that were associated with higher cortisol levels in pregnancy were lower maternal age [1.5 nmol/l, 95%CI (0.6 to 2.4)], being nulliparous [21.5 nmol/l (15.9 to 27.1)], lower pre-pregnancy body mass index (BMI) [1.3 nmol/l (0.3 to 2.4)], higher C-reactive protein (CRP) [1.0 nmol/l (0.4 to 1.5)], carrying a female fetus [9.2 nmol/l (1.8 to 16.5)], non-smoking [14.2 nmol/l (0.6 to 27.7)], sufficient sleep [8.5 nmol/l (0.9 to 16.1)], and being unemployed [12.7 nmol/l (2.2 to 23.2)]. None of the psychosocial stressors was significantly associated with serum cortisol levels in pregnancy. A total of 32% of all variance in cortisol was explained by gestational age, maternal age, time of day, parity, pre-pregnancy BMI, CRP, fetal sex, smoking behavior, self-reported sleep sufficiency, and employment.

Conclusions: Our data suggest that maternal cortisol during pregnancy is mainly affected by biological and lifestyle factors, but not by psychosocial factors. We suggest that psychosocial stress in

pregnancy might program the fetus through other mechanisms than through altering maternal cortisol levels.

Keywords: Prenatal stress, fetal programming, cortisol, HPA-axis, programming mechanisms, DoHaD

1. Introduction

An adverse intra-uterine environment may modify fetal neuroendocrine systems and metabolism, leading to a potentially increased risk of cardio metabolic, psychiatric and neurological disorders in later life (Whalley et al. 2006; Buss et al. 2012; Glover 2015a). One of the proposed underlying mechanisms is excessive fetal exposure to maternal glucocorticoids, produced by the hypothalamic-pituitary-adrenocortical (HPA) axis in response to physiological or psychological stress. The fetus is partly protected from cortisol exposure through deactivation by the placental enzyme 11-beta hydroxysteroid dehydrogenase (HSD-11β). However, active cortisol can still enter the fetal circulation (Seckl 2004), and alter the fetal HPA axis, as has been shown in animal studies (Seckl & Meaney 2004; Weinstock 2005).

Programming effects of both antenatal maternal mood (Glover 2015b) and glucocorticoids (Dalziel et al., 2005; Davis et al., 2011) in humans on offspring health and behavior have been described as well. A wealth of studies have shown that offspring of mothers with high levels of psychosocial stress, depression or anxiety are at increased risk for adverse neurodevelopmental outcomes, showing more internalizing and externalizing behavior problems (O'Connor et al. 2002; O'Connor et al. 2003; O'Donnell et al. 2014) higher symptoms of depression (Pawlby et al. 2009; Pearson et at 2013; Plant et al. 2013) and alterations in biological systems such as brain morphology (Buss et al. 2010) and

epigenetic profiles (Oberlander et al. 2016). Higher levels of cortisol during pregnancy have also been related to offspring IQ (LeWinn et al. 2009), behavior and temperament (de Weerth et al. 2013).

Maternal mood has been related to cortisol levels in earlier literature, showing associations of stress (Shelton et al. 2015), depression (Peer et al. 2013) and anxiety (Sarkar et al. 2006) with maternal cortisol. The proposed theory that stress-associated programming occurs through increased maternal cortisol levels and subsequently fetal exposure is therefore plausible. However, other studies have found only moderate correlations between prenatal stress, depression or anxiety and maternal cortisol levels (Wadhwa et al., 1996; Diego et al., 2006; Harville et al., 2009), or no correlation at all (Petraglia et al., 2001; Goedhart et al., 2010; Voegtline et al., 2013).

One possible explanation for this inconsistency in findings is that cortisol during pregnancy is affected by many factors other than stress. Cortisol has been associated with various biological factors. In the non-pregnant population, increasing age has been associated with higher cortisol levels (Laughlin & Barrett-Connor 2000; Nater et al. 2013) and inflammation parameters such as C-Reactive Protein (CRP) have been described to play an important role in cortisol regulation as well (Ottaviani & Franceschi 1998; Black 2003). Pregnancy itself has been stated a 'controlled inflammatory process' in which cortisol and CRP levels are correlated, allowing an appropriate environment to allow pregnancy, and dysregulation might result in adverse obstetric outcomes (Wilder 1998, Mor & Cardenas 2010). In pregnancy, higher cortisol levels were found in nulliparous women compared to multiparous women (Vleugels et al. 1986; Conde & Figueiredo 2014), and lower cortisol levels were reported in women with a higher body mass index (BMI) (Stirrat et al. 2016). Furthermore, environmental factors such as smoking behavior also appear to influence cortisol levels (Dušková et al. 2012). These studies altogether illustrate the multifaceted aspect of cortisol regulation. This is complicated even more by the fact that pregnancy in itself causes a physiological state of hypercortisolism, increasing with advancing gestation (Soma-Pillay et al. 2016).

To disentangle the independent contribution of determinants to maternal cortisol during pregnancy, we aimed to perform a study in a large cohort, using data from the Amsterdam Born Children and their Development (ABCD) cohort, with extensive data on maternal pregnancy characteristics. This study extends on a previous study describing associations between maternal cortisol and birthweight (Goedhart et al. 2010). The current study aimed to include several maternal biological, lifestyle and psychosocial factors in our analysis, possibly identify novel determinants, and assess the contribution of psychosocial stress when all other factors are taken into account. We looked at different subgroups to compare directions and effect sizes according to ethnicity, employment and parity, which may interact with HPA-axis activity (Vleugels et al. 1986; Rasheed 1993). We hypothesized that biological and environmental factors are more strongly associated with maternal cortisol levels than psychosocial factors, and that this may differ per subgroup.

2. Methods

This study was performed using data from the ABCD cohort, a prospective population-based cohort study examining associations between maternal pregnancy conditions and lifestyle with offspring health at birth and later in life (van Eijsden et al. 2011). Approval of the study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of Amsterdam, and was conducted according to the declaration of Helsinki. All participants gave written informed consent.

2.1. Selection of participants

Between January 2003 and March 2004, all pregnant women living in Amsterdam (n=12,373, covering an estimated ≥99% of the target population) were invited to participate in the ABCD study during their first prenatal visit to a general practitioner, midwife or gynaecologist. 8266 women (67%) returned a completed questionnaire that was sent to their homes, covering sociodemographic

characteristics, obstetric history, lifestyle and psychosocial conditions. Although relatively more women born from Dutch parents participated (77%) compared to foreign-born women (42-64%), response rates were comparable with other Dutch population-based studies including multiple ethnicities (van Eijsden et al. 2011). 4389 women participated in a biomarker study. A total of 1249 women (28%) were excluded because information on the time of day the blood sample was taken as well as on gestational age at that time was not available. This resulted in a group of 3140 women, of whom 3044 women gave birth to a live-born singleton. Five women used steroid medication and were therefore excluded, resulting in a sample size of 3039 women who were included for the current analysis. These women were older, more often nulliparous, more often of Dutch origin, more highly educated, more often employed, had lower BMI's, smoked less often and consumed alcohol more often compared to women in the original cohort.

2.2. Cortisol

One blood sample was taken in a 9-ml Vacuette (Greiner BV, Alphen aan de Rijn, the Netherlands) and processed at the Regional Laboratory of Amsterdam. 1-mL plasma and serum aliquots were first centrifuged and then stored at -80 ^{o}C until analysis was performed (mean: 22 months (range 13-29)) at the National Institute for Public Health and the Environment, Bilthoven, the Netherlands. Total cortisol in serum was determined by radio-immunoassay and defined in µg/L, and for the purpose of this paper converted to nmol/l by multiplying by 2.759. The interassay coefficient of variation (CV) was 10.2% for low values and 4.9% for high values (Goedhart et al. 2010).

2.3. Time of day and gestational age

The time of day and gestational age at the moment of blood sampling were obtained from pregnancy records.

2.4. Biological factors

Women completed a pregnancy questionnaire at a mean gestational age of 114 days (SD= 26 days). A total of 53 women completed the questionnaire before the blood sample was taken (mean= -12 days, SD= -8 days), and 2986 women completed the questionnaire after the blood sample was taken (mean=21 days, SD=17 days). Characteristics reported by the pregnant women included maternal age, parity and ethnicity (defined as the country of birth of the mother of the pregnant women). CRP was measured in blood serum. The sex of the unborn child was obtained from the register of the Youth Health Department at the Municipal Health Service Amsterdam. Women reported on pre-existing diseases, according to the International Classification of Primary Care (ICPC: https://www.nhg.org/themas/publicaties/icpc-online), as well as pregnancy-related diseases (including sexually transmitted disorders, gestational diabetes, pregnancy-induced hypertension, vaginal blood loss, anemia and rubella) (Yes/No/Don't know). Pre-pregnancy BMI was calculated by dividing self-reported pre-pregnancy weight by the square root of height. Women reported on nausea or vomiting for 2 weeks or more (Yes/No) and if they had lost 5 kilograms or more during pregnancy (Yes/No).

2.5. Lifestyle factors

Women reported on smoking and alcohol consumption during the past weeks (No/1 cigarette or unit per day/>1 cigarettes or units per day). Socio-economic status was defined as years of education after primary school. Women reported whether they currently had a paid job or not (Yes/No). Women reported on the use of medication before pregnancy according to the Anatomical Therapeutic Chemical classification (ATC: http://www.whocc.no/atc_ddd_index/), and the use of medication during pregnancy (Yes/No). Women were asked if they perceived the number of hours they slept (Sufficient/Too long/Too little) and if they had performed (one of) the following exercises during the past week: hiking, cycling, going to the gym and-/or other forms of physical exercise (Yes/No).

2.6. Stress factors

To determine pregnancy related anxiety, a 10-item questionnaire, based on the Pregnancy-Related Anxiety Questionnaire-Revised (PRAQ-10) was used. The validated Dutch version of the 20-item Center for Epidemiologic Studies Depression (CES-D) screening test was used to assess depressive symptoms. To assess anxiety symptoms, the validated Dutch version of the State-Trait Anxiety Inventory (STAI) was used, involving 20 items. The validated Dutch translation of the Frequency scale of the Parenting Daily Hassles (PDH) was used, consisting of 20 events that typically occur in families with young children. Job strain was assessed using a validated Dutch questionnaire based on the Job Content Questionnaire (JCQ), consisting of 25 items on job demand and 11 items on job control. To assess self-perceived fatigue, the Multidimensional Fatigue Inventory (MFI) was used, which was originally developed and validated in the Dutch language. The MFI is a self-report questionnaire designed to measure fatigue (Smets et al. 1995). For complete references, we refer to the paper of Goedhart (2010). All questionnaires showed excellent reliability (Cronbach's alpha ≥ 0.8).

2.7. Statistical Analysis

SPSS statistics version 23 (IBM) was used for the analysis of data. Visual inspection of cortisol values indicated a normal distribution and analyses were performed with raw data. To be able to include participants with missing data on one or more of the questions of the stress-questionnaires (PRAQ: n=57 (2%), fatigue: n=90 (3%), Job Demand: n= 96 (4%), Job Control: n=26 (1%), parenting hassles: n=120 (9%) CES-D: n=160 (5%), STAI: n=116 (4%)), regardless of the number of questions that had been answered (with a minimum of 1), average scores of the stress-related scores were calculated by dividing the total score by the amount of questions that were completed per person. This individual mean imputation method has shown to be a simple and appropriate method when the number of missing data is low (Shrive et al., 2006). For continuous variables, Spearman's correlations between cortisol and maternal characteristics were calculated, and for dichotomous variables, Spearman's correlations were calculated. Ethnicity was dichotomized (Dutch/not-Dutch) for this purpose.

Correlations showing a correlation coefficient (r) higher than 0.3 or lower than -0.3 were reported. Associations between all independent variables and cortisol were calculated using a linear regression model. Linearity of the associations was checked by visual inspection of the scatterplots. All independent variables were either dichotomous (%) or continuous (Mean, SD), categorical (%) variables were recoded into dummy variables (ethnicity) or dichotomous (Yes/No) variables (smoking, drinking behavior, medication use, diseases, sleeping satisfaction, and exercise). We reported unadjusted univariate regression analyses and regression analyses adjusted in two steps. In model 1, we adjusted for time of day (continuous variable, coded as '1' for samples taken between 08:00 and 09:00 am, as '2' for samples taken between 09:00 and 10:00 am, and so on, with a maximum of '11' for samples taken after 06:00 pm), and gestational age (continuous variable, in days). In model 2, we additionally adjusted for ethnicity and employment status regardless of their independent association with cortisol, and we included all independent variables that showed an association of $p<0.1$ in the first model.

Subsequent subgroup analyses were performed according to parity (nulliparous and multiparous), employment (having a paid job or not) and ethnicity (Dutch, Creole, Turkish, Moroccan or Other) adjusted for time of day, gestational age, and all the variables that showed a significant association with cortisol in the total study sample. We included parenting hassles in the subgroup analysis of multiparous women only and job strain in the subgroup analysis of working women only. We analyzed ethnic subgroups, using the ethnicities that were represented most often in our study sample. We additionally performed a sensitivity-analysis in all women who were in their second trimester (between 92 and 189 gestational days) of pregnancy and whose cortisol sample was collected between 11:00 and 12:00 am, representing the hour in which the relatively largest amount of blood samples was taken (n=600), to assess the associations with less potential confounding effects of the time of day and pregnancy duration on cortisol values. We used interaction terms to test potential interactions by multiplying the centered independent variables with the potential

centered moderators, and including these in a linear regression model with cortisol as dependent variable.

3. Results

3.1. General characteristics

Characteristics of the participants are shown in table 1. The mean cortisol level of the study sample (n=3039) was 337 nmol/l (SD=124), with a mean gestational age of 94 days (SD=21), when cortisol was measured. Cortisol values of women collected in later gestation were higher (fig. 1) and those collected later in the day were lower (fig.2).

3.2. Correlations with cortisol

Gestational age correlated positively with cortisol levels ($r=0.3$, $p<0.001$) and negatively with time of day ($r=-0.4$, $p<0.001$). No other variable showed a correlation coefficient (r) higher than 0.3 or lower than -0.3 with maternal cortisol.

3.3. Correlations between all measures

Older women had more often higher parity ($r=0.3$, $p<0.001$). Women who were Dutch ($r=0.4$, $p<0.001$) and women who were older ($r=0.3$, $p<0.001$) had more years of education. Dutch women were more often employed ($r=0.4$, $p<0.001$). Women with more parenting hassles experienced higher levels of anxiety ($r=0.3$, $p<0.001$) and depression ($r=0.4$, $p<0.001$), as did women with higher pregnancy-related anxiety ($r=0.4$, $p<0.001$ for anxiety, and $r=0.3$, $p<0.001$ for depression). Women who reported to get the amount of sleep they needed reported less fatigue than those who would prefer to sleep more or less ($r=0.4$, $p<0.001$). Women who reported more depressive ($r=0.6$, $p<0.001$) or anxiety ($r=0.5$, $p<0.001$) symptoms reported higher fatigue. Women who preferred more

or less sleep also reported higher depression and anxiety symptoms (both r=0.3, p<0.001). Depressive and anxiety symptoms were strongly correlated (r=0.8, p<0.001).

3.4. Regression analysis

Table 2 shows the univariate regression results of all independent variables in relation to cortisol in all women (n=3039). Multivariate model 1, corrected for time of day and gestational age, shows that higher cortisol was associated with lower maternal age [2.7 nmol/l, 95%CI (1.9 to 3.5)], being nulliparous [23.5 nmol/l (18.9 to 28.2)], lower pre-pregnancy BMI [1.9 nmol/l (0.9 to 2.9)], higher CRP levels [0.5 nmol/l (-0.1 to 1.0)], carrying a female fetus [8.4 nmol/l (1.0 to 15.8)], non-smoking [13.2 nmol/l (-0.2 to 26.6)], self-reported sufficient sleep [11.7 nmol/l (4.2 to 19.3)], and higher pregnancy-related anxiety [10.8 nmol/l (2.9 to 18.8)], which were subsequently added to model 2 (n=2974), in which we also corrected for ethnicity, and employment. All directions and effect sizes remained similar and significant, with the exception of pregnancy related anxiety. The total explained variance of the fully adjusted model was 32%.

3.5. Subgroup analyses

Results of the subgroup analyses are presented in table 3.

3.5.1. Nulliparous and multiparous women

There was no association between parenting hassles and cortisol in multiparous women. Interactions with parity and cortisol were found for maternal age (p=0.01), pre-pregnancy BMI (p=0.01), and employment (p=0.002), indicating that the effects of higher maternal age, higher pre-pregnancy BMI and being employed on lower cortisol levels were greater in nulliparous women (n=1715) compared to multiparous women (n=1285).

3.5.2. Employed and unemployed women

There was no association between job hassles and cortisol in employed women. Interactions with employment and cortisol were seen for maternal age (p=0.03) and parity (p=0.002), indicating that the effect of higher maternal age and higher parity on lower cortisol levels was greater in unemployed women compared to employed women. Another interaction was seen with employment and cortisol for pregnancy-related anxiety (p=0.04), showing that higher levels of pregnancy-related anxiety associated with lower cortisol levels in employed women (n=2196), but with higher cortisol levels in unemployed women (n=613).

3.5.3. Women with different ethnicities

We performed subgroup analysis in five different ethnic groups. In Dutch women (n=1901), an interaction with cortisol was seen for fetal sex (p=0.03), indicating that the effect of carrying a female fetus on higher cortisol levels was smaller in Dutch women compared to non-Dutch women. In Creole women (n=165), an interaction with cortisol was seen for parity (p=0.04), indicating that the effect of higher parity on lower cortisol was smaller in Creole women compared to non-Creole women. In Turkish women (n=114), interactions with cortisol were seen for CRP (p=0.01) indicating that the effect of higher CPR on cortisol was negative only in Turkish women, whereas in non-Turkish women this effect was positive. In Moroccan women (n=165), interactions with cortisol were seen for maternal age (p=0.04), showing that only in Moroccan women, higher maternal age was associated with higher cortisol levels. Also, an interaction with cortisol was seen for self-reported sufficient sleep (p=0.04), showing that the effect of insufficient sleep on lower cortisol levels was greater in Moroccan women than in non-Moroccan women. In both Moroccan and Turkish women, interactions with cortisol for parity were found (p=0.002 and p=0.003 respectively), showing that the effect of higher parity on lower cortisol was greater in both Moroccan and Turkish women compared to women of other ethnic backgrounds.

3.6. Sensitivity analysis

A sensitivity analysis was performed in a subsample of 600 women, in whom cortisol was measured between 11:00 and 12:00 am in the second trimester. In this group, cortisol ($p<0.001$) and CRP ($p<0.05$) had higher values compared to the total study sample. The group did not differ in terms of maternal age, BMI, fetal sex, parity ethnicity, smoking, alcohol use, education level, medication use, diseases, exercise or stress measures compared to the total study sample. The regression model included maternal age, parity, pre-pregnancy BMI, CRP, fetal sex, smoking, sleeping behavior, employment and pregnancy-related anxiety as dependent variables and cortisol as the outcome variable. Directions of the effects of maternal age, parity, BMI and insufficient sleep were in line with the total study sample. Only CRP, smoking and employment status was less clearly associated with cortisol. Pregnancy related anxiety was not associated with cortisol.

4. Discussion

In this explorative study, we aimed to identify determinants of women's cortisol levels during pregnancy in a large cohort. Our data suggest that maternal cortisol during pregnancy is affected mainly by biological and lifestyle factors, and that psychosocial stress does not seem to play a significant role when the other factors are taken into account.

Biological factors

Cortisol levels decreased linearly with increasing age, which is contrary to findings in the non-pregnant population (Laughlin & Barrett-Connor 2000; Nater et al. 2013). This might be explained by the relative youthfulness of our sample, ranging from 16 to 44 years of age, in which the proposed 'age-related increase in HPA axis activity' is not apparent yet. We also found higher cortisol levels in nulliparous women compared to multiparous women, which is a replication of earlier findings (Vleugels et al. 1986; Conde & Figueiredo 2014). Self-reported pre-pregnancy BMI was inversely

associated with cortisol. Some recent studies report similar findings (Berglund et al. 2016; Stirrat et al. 2014), and suggest this may underlie the association between higher BMI during pregnancy with macrosomia and prolonged gestation.

We found that women carrying female fetuses had higher cortisol levels than women carrying males, in accordance with earlier literature (DiPietro et al. 2011). One explanation is possible maturational delay in male fetuses compared to females (Buss et al. 2009), or different growth strategies, with female fetuses limiting growth in benefit of maintaining resource reserves (Clifton 2010). Different immune function in women carrying a female fetus has also been described, showing women carrying female fetuses have greater stimulated production of interleukin(L)-6, tumor necrosis factor(TNF)- α and IL-1-β (Mitchell et al. 2017) and greater susceptibility to asthma exacerbations (Bakhireva et al. 2008). These findings may contribute to the growing body of literature investigating sexual dimorphisms in pregnancy outcomes, and imply that taking fetal sex into account might be important when determining obstetric risks.

CRP levels were positively associated with cortisol levels. A handful of earlier studies do suggest that CRP and cortisol are closely linked. CRP is a biological marker of infection, but is also elevated during pregnancy (Maguire et al. 2015) to enhance a finely-balanced 'controlled inflammatory process', enabling implantation, placentation and discharge of the baby (Mor & Cardenas 2010). It has been described that this process is cortisol-regulated, which if disrupted might result in over-activation of the inflammatory system (Wilder 1998), with potential risks for mother and child.

Lifestyle factors

Smoking was inversely associated with cortisol levels. In two earlier studies, opposite findings have been described (Obel et al. 2005; Lopez & Seng 2014). However, one of these studies included only 29 smoking women, and both studies measured cortisol levels in saliva, which might explain the difference in findings. Our finding may be a reflection of a potential underlying pathway from

smoking during pregnancy to a reduction in static compliance of the lungs in boys and conductance in girls, since cortisol enhances lung ripening (Milner et al. 1999).

Women who reported to perceive the duration of their sleep as too long or too little had lower cortisol values compared to women who reported sufficient sleep. These results coincide with findings from a similar (but small) study suggesting alterations of the circadian pacemaker system of poor sleepers, in which lower cortisol area under the curve values were associated with worse sleep quality (Crowley et al. 2016).

Finally, in our study sample, employed pregnant women had lower cortisol levels than unemployed pregnant women. One earlier study also found relatively higher morning and evening levels of cortisol in unemployed participants (Ockenfels et al. 1995). Whereas they propose a mediating role of depressive symptoms in unemployed subjects, our findings do not support hypothesis.

Psychosocial stress

None of the psychosocial stressors were associated with maternal cortisol, except for pregnancy-related anxiety. However, this factor did not survive full adjustment suggesting that other factors influencing pregnancy-related anxiety are responsible for the changes in cortisol levels. This is in contrast with earlier evidence describing associations between antenatal stress, depression, and anxiety with maternal cortisol levels (Sarkar 2006; Peer et al. 2013; Shelton et al. 2015). The absence of independent associations between maternal psychosocial stress and cortisol is striking especially since cortisol has been put forward as such an important mechanism in developmental programming of later life health by maternal stress. It must be noted that women completed the psychosocial stress questionnaires several days to weeks before or after their cortisol was measured, potentially resulting in inaccurate results in women who would have responded differently at the day of sampling. However, our findings do correspond with quite a few studies showing rather modest correlation coefficients (Wadhwa et al., 1996; Diego et al., 2006) , or no relationship at all (Harville et

al. 2009; Voegtline et al. 2013). The lack of findings might be explained by the fact that, despite a wide range in gestational ages, most women in our study sample were in their first trimester of pregnancy, and different effects of perceived stress on cortisol have been described across the course of pregnancy (Obel et al. 2005). Also, anxiety and depressive symptoms differ between trimesters, although they are particularly high during the first trimester in primiparous women, which is the majority of our cohort (Texeira et al. 2009). Also, cortisol may simply not be the most accurate marker of perceived psychological stress. The lack of an association between cortisol levels and perceived stress has been demonstrated in the general population before. One systematic review showed that the evidence for a relation between perceived stress and activity of the endocrine system is not convincing (Dawe et al. 2016), and another systematic review found that in 73% of the included studies, there was no significant association between subjective stress and cortisol in response to the Trier Social Stress Test (TSST), which has been shown to reliably induce an increase in cortisol (Campbell & Ehlert 2012). Campbell and Ehlert give a nice overview of potential explanations for this discrepancy, including among others the validity of stress questionnaires, timing of measurements and differences in appraisal processes and emotion regulation (Campbell & Ehlert 2012). An alternative explanation is that subjective psychosocial stress only significantly correlates with cortisol in cases of severe stress, whereas in our healthy study sample, the levels of stress were generally low.

Psychosocial factors may still affect women's physiology resulting in fetal programming effects, but through other or intermediate mechanisms than exerting a direct effect on maternal cortisol. Zijlmans et al reviewed 29 studies and found little evidence supporting the hypothesis that maternal cortisol underlies the association between maternal prenatal stress and health outcomes in the child (Zijlmans et al. 2015). Possibly, psychosocial stress increases placental cortisol *transfer* through a decreased placental HSD-2 activity, as shown in women with higher anxiety symptoms (Glover et al. 2009). Other potential underlying mechanistic pathways are reduced uterine blood flow, changes in the immune and central nervous system or epigenetic changes induced by stress.

Subgroups

We evaluated different subgroups in our dataset to compare effect sizes and directions. Effects of maternal age, pre-pregnancy BMI and employment on cortisol seemed to be larger among nulliparous than among multiparous women. To our knowledge, this difference in effects between nulliparous and multiparous women has not been described before. Our findings possibly reflect a more reactive HPA-axis in nulliparous women, which might underlie the increased risk for maternal and obstetric complications in nulliparous women as described earlier in this section (Phocas et al. 1990).

It must be noted that in the subgroup analyses, ethnicity is likely to play a role. Dutch women were more often employed and nulliparous, non-Dutch women had higher gestational ages at their first pregnancy visit, were younger and had higher BMI's. Although we adjusted for these factors, the variation in demographics combined with relatively small sample sizes of non-Dutch women, complicates interpretation of our findings. In our subgroup analysis, according to ethnicity, we found that ethnicity seems to moderate the effect of fetal sex, parity, CRP, self-reported insufficient sleep and pre-pregnancy BM on cortisol values. For the self-reported variables BMI and sleeping behavior, this might be explained by different objective interpretations in different ethnic groups. The interaction with parity might be due to the fact that women with Turkish and Moroccan background more often had had more than two pregnancies than women with a Dutch ethnicity. If the decrease in cortisol values after the first pregnancy continues with every subsequent pregnancy, this might explain the fact that in these women, parity had such a strong effect on cortisol. The interaction with age and CRP might be explained by the fact that women of non-Dutch ethnicity were generally younger, and had higher CRP values.

Implications & recommendations

Our study results have some reassuring implications for clinicians as well as healthy pregnant women who are concerned about the impact of maternal stress on cortisol levels and in turn fetal development. However, we cannot rule out programming effects through alternative underlying mechanisms linking maternal stress to altered fetal phenotypes. Therefore, these implications are to be interpreted with caution. In future studies cortisol should be measured at several stages of pregnancy, at a consistent time during the day, and with concurrent measures of psychosocial stress. Also, alternative mechanisms of fetal programming through prenatal stress should be addressed.

Strengths and limitations

A strength of our study is the large sample size and detailed information available for pregnant women, enabling us to include several variables in our linear regression model. However, our study sample consisted of only 25% of the initial cohort, after exclusion of women without cortisol measures and women for whom no time of day and gestational age during blood sampling was known. Compared to the original group, these women were older, more often nulliparous, more often of Dutch origin, more highly educated, more often employed, had lower BMI's, smoked less often and consumed alcohol more often compared to the original cohort, which is therefore not fully representative of the pregnant population in Amsterdam. However, in a previous study in this cohort we found no effect of attrition bias when taking these differences into account. Also, an additional analysis with imputation for missing data on time of day and gestational age, increasing the sample size to 35% of the initial cohort, did not change the results (data not shown). Another limitation of the study is the variability between the participants in the time of day and gestational age when cortisol was measured, as well as the time gap between cortisol and psychosocial measurements. Concurrently, we showed the well-known patterns of increase with advancing pregnancy (Soma-Pillay et al. 2016) (figure 1), and the circadian rhythm of the HPA-axis, resulting in a peak of cortisol in the morning, followed by a consistent decrease during the day (Allolio et al. 1990) (figure 2). We performed a sensitivity analysis, restricting the variability in gestational age and timing, focusing on

women in whom samples were taken during the second trimester between 11 and 12 am. Cortisol associations with biological factors remained, but those with environmental factors were less apparent. Cortisol levels were high in the sensitivity analysis subsample, with a mean of 386 nmol/l, which might conceal the subtle changes that are caused by lifestyle factors. A final limitation may be that we measured serum cortisol, which is a measure of total cortisol in the body. Others have found that free cortisol (as measured in saliva) was associated with stressful life events during specific trimesters of pregnancy (Obel et al. 2005)

5. Conclusion

Our data suggest that maternal serum cortisol levels during pregnancy are affected mostly by biological and to a lesser extent lifestyle factors, and that psychosocial stress factors do not seem to play a significant role when the other factors are taken into account. We suggest that prenatal stress might program offspring outcomes though cortisol levels only in cases of very severe stress, or possibly through other mechanisms influencing transfer of cortisol to the fetus than altered maternal serum cortisol levels.

Contributors

LB, SdR, TR, RR and TV were involved in study design. TV was involved in the data collection. LB, SdR and TR were involved in data analysis. LB prepared a draft of the manuscript, which was reviewed and approved by all co-authors. SdR checked the manuscript for grammar and stylistic correctness. All authors approved the final manuscript and its submission to Psychoneuroendocrinology

Role of funding source

Laura Bleker and Susanne de Rooij have been funded by project DynaHealth: *Understanding the*

Laura S. Bleker

Dynamic determinants of glucose homeostasis and psychological capacity to promote healthy and active ageing under Grant Agreement no 633595 (Horizon2020). The funding organizations are public institutions and had no role in the design and conduct of the study; collection, management, and analysis of the data; or preparation, review, and approval of the manuscript.

Conflicts of interest

None declared.

Acknowledgement

The ABCD study was financially supported by a research grant from the Netherlands Organization of Health Research and Development (grant number 21000076). We are grateful to all participating hospitals, obstetric clinics and general practitioners for their assistance in implementing the ABCD study, and we thank all the women who participated for their cooperation. RR acknowledges support of the British Heart Foundation and Tommys. TJR acknowledges support for the Dutch Heart Foundation (2013T085).

Laura S. Bleker

References

Allolio, B. et al., 1990. Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing-hormone. *Clinical endocrinology*, 33(2), pp.279–89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2225483 [Accessed November 22, 2016].

Bakhireva LN, Schatz M, Jones KL et al., 2008. Fetal seks and maternal asthma control in pregnancy, *Journal of asthma,* 45(5), p.403-7. Available at: https://www.ncbi.nlm.nih.gov/pubmed/18569234 [Accessed March, 2017].

Berglund, S.K. et al., 2016. Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). *BMC public health*, 16, p.207. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26931143 [Accessed November 22, 2016].

Black, P.H., 2003. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain, behavior, and immunity*, 17(5), pp.350–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12946657 [Accessed November 22, 2016].

Buss, C. et al., 2009. Maturation of the human fetal startle response: evidence for sex-specific maturation of the human fetus. *Early human development*, 85(10), pp.633–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19726143 [Accessed November 22, 2016].

Buss C, Davis EP, Muftuler LT, 2010. High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6-9 year old children. *Psychoneuroendocrinology*, 35(1), pp.141-53. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19674845 [Accessed March 28, 2017].

Buss, C., Entringer, S. & Wadhwa, P.D., 2012. Fetal programming of brain development: intrauterine stress and susceptibility to psychopathology. *Science signaling*, 5(245), p.pt7. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/23047922 [Accessed November 22, 2016].

Campbell, J. & Ehlert, U., 2012. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology*, 37(8), pp.1111–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22260938 [Accessed November 22, 2016].

Clifton, V.L., 2010. Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta*, 31 Suppl, pp.S33-9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20004469 [Accessed November 22, 2016].

Conde, A. & Figueiredo, B., 2014. 24-h urinary free cortisol from mid-pregnancy to 3-months postpartum: gender and parity differences and effects. *Psychoneuroendocrinology*, 50, pp.264–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25247747 [Accessed November 22, 2016].

Crowley, S.K. et al., 2016. Blunted neuroactive steroid and HPA axis responses to stress are associated with reduced sleep quality and negative affect in pregnancy: a pilot study. *Psychopharmacology*, 233(7), pp.1299–310. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26856852 [Accessed November 22, 2016].

Dalziel, S.R. et al., Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. *Lancet (London, England)*, 365(9474), pp.1856–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15924982 [Accessed November 22, 2016].

Davis, E.P. et al., 2011. Prenatal maternal stress programs infant stress regulation. *Journal of child psychology and psychiatry, and allied disciplines*, 52(2), pp.119–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20854366 [Accessed November 22, 2016].

Dawe, K. et al., 2016. The effects of perceived stress on biological parameters in healthcare professionals: A systematic review. *Journal of health psychology*, 21(5), pp.607–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24829376 [Accessed November 22, 2016].

Diego, M.A. et al., Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosomatic*

medicine, 68(5), pp.747–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17012528 [Accessed November 22, 2016].

DiPietro, J.A. et al., 2011. Maternal salivary cortisol differs by fetal sex during the second half of pregnancy. *Psychoneuroendocrinology*, 36(4), pp.588–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20940089 [Accessed November 22, 2016].

Dušková, M. et al., 2012. Chronic cigarette smoking alters circulating sex hormones and neuroactive steroids in premenopausal women. *Physiological research*, 61(1), pp.97–111. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22188108 [Accessed November 22, 2016].

van Eijsden, M. et al., 2011. Cohort profile: the Amsterdam Born Children and their Development (ABCD) study. *International journal of epidemiology*, 40(5), pp.1176–86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20813863 [Accessed November 22, 2016].

Glover, V. et al., 2009. Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. *Psychoneuroendocrinology*, 34(3), pp.430–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19019559 [Accessed November 22, 2016].

Glover, V., 2015a. Prenatal Stress and Its Effects on the Fetus and the Child: Possible Underlying Biological Mechanisms. In pp. 269–283. Available at: http://link.springer.com/10.1007/978-1-4939-1372-5_13 [Accessed November 22, 2016].

Glover, V., 2015b. Prenatal Stress and Its Effects on the Fetus and the Child: Possible Underlying Biological Mechanisms. In pp. 269–283. Available at: http://link.springer.com/10.1007/978-1-4939-1372-5_13 [Accessed November 7, 2016].

Goedhart, G. et al., 2010. Maternal cortisol and offspring birthweight: results from a large prospective cohort study. *Psychoneuroendocrinology*, 35(5), pp.644–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19889503 [Accessed November 22, 2016].

Harville, E.W. et al., 2009. Stress questionnaires and stress biomarkers during pregnancy. *Journal of women's health (2002)*, 18(9), pp.1425–33. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/19757520 [Accessed November 22, 2016].

Laughlin, G.A. & Barrett-Connor, E., 2000. Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo Study. *The Journal of clinical endocrinology and metabolism*, 85(10), pp.3561–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11061502 [Accessed November 22, 2016].

LeWinn KZ, Stroud LR, Moinar BE, 2009. Elevated maternal cortisol levels during pregnancy are associated with reduced childhood IQ, *International Journal of Epidemiology,* 38(6), pp. 1700-10. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19423658 [Accessed March 28, 2017.]

Lopez, W.D. & Seng, J.S., 2014. Posttraumatic stress disorder, smoking, and cortisol in a community sample of pregnant women. *Addictive behaviors*, 39(10), pp.1408–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24926909 [Accessed November 22, 2016].

Maguire, P.J. et al., 2015. Maternal C-reactive protein in early pregnancy. *European journal of obstetrics, gynecology, and reproductive biology*, 193, pp.79–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26254855 [Accessed November 22, 2016].

Milner, A.D. et al., 1999. Effects of smoking in pregnancy on neonatal lung function. *Archives of disease in childhood. Fetal and neonatal edition*, 80(1), pp.F8-14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10325804 [Accessed December 2, 2016].

Mitchell AM, Palettas M, Christian LM et al., 2017. Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy, Brain Behaviour and Immunity, pp.32-37. Available at https://www.ncbi.nlm.nih.gov/pubmed/27375004 [Accessed March 28, 2017].

Mor, G. & Cardenas, I., 2010. The immune system in pregnancy: a unique complexity. *American journal of reproductive immunology (New York, N.Y. : 1989)*, 63(6), pp.425–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20367629 [Accessed November 22, 2016].

Nater, U.M., Hoppmann, C.A. & Scott, S.B., 2013. Diurnal profiles of salivary cortisol and alpha-

amylase change across the adult lifespan: evidence from repeated daily life assessments. *Psychoneuroendocrinology*, 38(12), pp.3167–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24099860 [Accessed November 22, 2016].

Obel, C. et al., 2005. Stress and salivary cortisol during pregnancy. *Psychoneuroendocrinology*, 30(7), pp.647–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15854781 [Accessed November 22, 2016].

Oberlander TF, Weinberg J, Papsdorf M, et al, 2016. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 3(2), pp.97-106. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18536531. [Accessed March 28, 2017.]

Ockenfels, M.C. et al., Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosomatic medicine*, 57(5), pp.460–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8552737 [Accessed November 22, 2016].

O'Connor TG, Heron J, Golding J, et al, 2003. Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. *Journal of child psychology and psychiatry, and allied disciplines,* 44(7), pp.1025-36. Available at: https://www.ncbi.nlm.nih.gov/pubmed/26104631 [Accessed March 28, 2017].

O'Connor TG, Heron J, Golding J, et al, 2012. Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. *The British journal of psychiatry : the journal of mental science,* 180, pp.502-8. Available at: https://www.ncbi.nlm.nih.gov/pubmed/12042228 [Accessed March 28, 2017].

O'Donnell KJ, Glover V, Barker ED, et al,2014. The persisting effect of maternal mood in pregnancy on childhood psychopathology. *Development and psychopathology*, 26(2), pp.393-403. Available

at: https://www.ncbi.nlm.nih.gov/pubmed/24621564 [Accessed March 28, 2017].

Ottaviani, E. & Franceschi, C., 1998. A new theory on the common evolutionary origin of natural immunity, inflammation and stress response: the invertebrate phagocytic immunocyte as an eye-witness. *Domestic animal endocrinology*, 15(5), pp.291–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9785032 [Accessed November 22, 2016].

Pawlby S, Hay DF, Sharp D, et al, 2009. Antenatal depression predicts depression in adolescent offspring: prospective longitudinal community-based study. *Journal of affective disorders,* 113(3), pp.236-43. Available at: https://www.ncbi.nlm.nih.gov/pubmed/18602698 [Accessed March 28, 2017].

Pearson RM, Evans J, Kounali D, et al,2013. Maternal depression during pregnancy and the postnatal period: risks and possible mechanisms for offspring depression at age 18 years, *JAMA psychiatry* 70(12), pp.1312-9. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24108418 [Accessed November 22, 2016].

Peer M, Soares CN, Levitan RD et al, 2013. Antenatal depression in a multi-ethnic, community sample of Canadian immigrants: psychosocial correlated and hypothalamic-pituitary-adrenal axis function. *Canada Journal of Psychiatry*, 58(10), p.579-88. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24165105 [Accessed March 28, 2017].

Petraglia, F. et al., Lack of effect of psychosocial stress on maternal corticotropin-releasing factor and catecholamine levels at 28 weeks' gestation. *Journal of the Society for Gynecologic Investigation*, 8(2), pp.83–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11336878 [Accessed November 22, 2016].

Phocas, I., Sarandakou, A. & Rizos, D., 1990. Maternal serum total cortisol levels in normal and pathologic pregnancies. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*, 31(1), pp.3–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1968015 [Accessed November 22, 2016].

Plant DT, Pariante CM, Sharp D, et al, 2015. Maternal depression during pregnancy and offspring depression in adulthood: role of child maltreatment. *The British Journal of Psychiatry,* 207(3), pp.213-20. Available at: https://www.ncbi.nlm.nih.gov/pubmed/26045352 [Accessed March 28, 2017].

Rasheed, F.N., 1993. Parity, birthweight, and cortisol. *Lancet (London, England)*, 341(8848), p.828. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8096027 [Accessed November 22, 2016].

Sarkar, P. et al., 2006. Maternal anxiety at amniocentesis and plasma cortisol. *Prenatal diagnosis*, 26(6), pp.505–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16683297 [Accessed November 22, 2016].

Seckl, J.R., 2004. Prenatal glucocorticoids and long-term programming. *European journal of endocrinology*, pp.U49-62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15554887 [Accessed November 22, 2016].

Seckl, J.R. & Meaney, M.J., 2004. Glucocorticoid programming. *Annals of the New York Academy of Sciences*, 1032, pp.63–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15677396 [Accessed November 22, 2016].

Shelton MM, Schminkey DL, Groer MW, 2014. Relationships among prenatal depression, plasma cortisol, and inflammatory cytokines. *Biological Research for Nursing,* 17(3), p.295-302. Available at: https://www.ncbi.nlm.nih.gov/pubmed/25230746 [Accessed March 28, 2017].

Shrive FM, Stuart H, Quan H et al., 2006. Dealing with missing data in a multi-question depression scale: a comparison of imputation methods. *BMC Medical Research Methodology*, 13;6;57. Abailable at: https://www.ncbi.nlm.nih.gov/pubmed/17166270 [Accessed March 28, 2017]

Smets, E.M. et al., 1995. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *Journal of psychosomatic research*, 39(3), pp.315–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7636775 [Accessed November 22, 2016].

Soma-Pillay, P. et al., 2016. Physiological changes in pregnancy. *Cardiovascular journal of Africa*,

27(2), pp.89–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27213856 [Accessed December 2, 2016].

Stirrat, L.I. et al., 2014. Altered maternal hypothalamic-pituitary-adrenal axis activity in obese pregnancy is associated with macrosomia and prolonged pregnancy. *Pregnancy hypertension*, 4(3), p.238. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26104631 [Accessed November 22, 2016].

Stirrat, L.I. et al., 2016. Decreased maternal hypothalamic-pituitary-adrenal axis activity in very severely obese pregnancy: Associations with birthweight and gestation at delivery. *Psychoneuroendocrinology*, 63, pp.135–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26444587 [Accessed November 22, 2016].

Texeira C, Figueiredo B, Conde A. et al, 2009. Anxiety and depression during pregnancy in women and men, *Journal of affective disorders,* 119(1-3), pp. 142-8. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19346001 [Accessed March 28, 2017]

Vleugels, M.P. et al., 1986. Cortisol levels in human pregnancy in relation to parity and age. *American journal of obstetrics and gynecology*, 155(1), pp.118–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3728579 [Accessed November 22, 2016].

Voegtline, K.M. et al., 2013. Concurrent levels of maternal salivary cortisol are unrelated to self-reported psychological measures in low-risk pregnant women. *Archives of women's mental health*, 16(2), pp.101–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23269500 [Accessed November 22, 2016].

Wadhwa, P.D. et al., Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. *Psychosomatic medicine*, 58(5), pp.432–46. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8902895 [Accessed November 22, 2016].

Weerth, de C., Buitelaar JK, Beijer R. Infant cortisol and behavioral habituation to weekly maternal separation: links with maternal prenatal cortisol and psychosocial stress,

psychoneuroendocrionology, 38(12), pp. 2863-74. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24103888 [Accessed March 28, 2017].

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, behavior, and immunity*, 19(4), pp.296–308. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15944068 [Accessed November 22, 2016].

Whalley, L.J., Dick, F.D. & McNeill, G., 2006. A life-course approach to the aetiology of late-onset dementias. *The Lancet. Neurology*, 5(1), pp.87–96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16361026 [Accessed November 22, 2016].

Wilder, R.L., 1998. Hormones, pregnancy, and autoimmune diseases. *Annals of the New York Academy of Sciences*, 840, pp.45–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9629235 [Accessed November 22, 2016].

Zijlmans, M.A.C., Riksen-Walraven, J.M. & de Weerth, C., 2015. Associations between maternal prenatal cortisol concentrations and child outcomes: A systematic review. *Neuroscience & Biobehavioral Reviews*, 53, pp.1–24. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0149763415000639 [Accessed November 22, 2016].

Figure 1. Scatterplot of values of plasma cortisol (nmol/l) at different gestational ages (days) (n=3039).

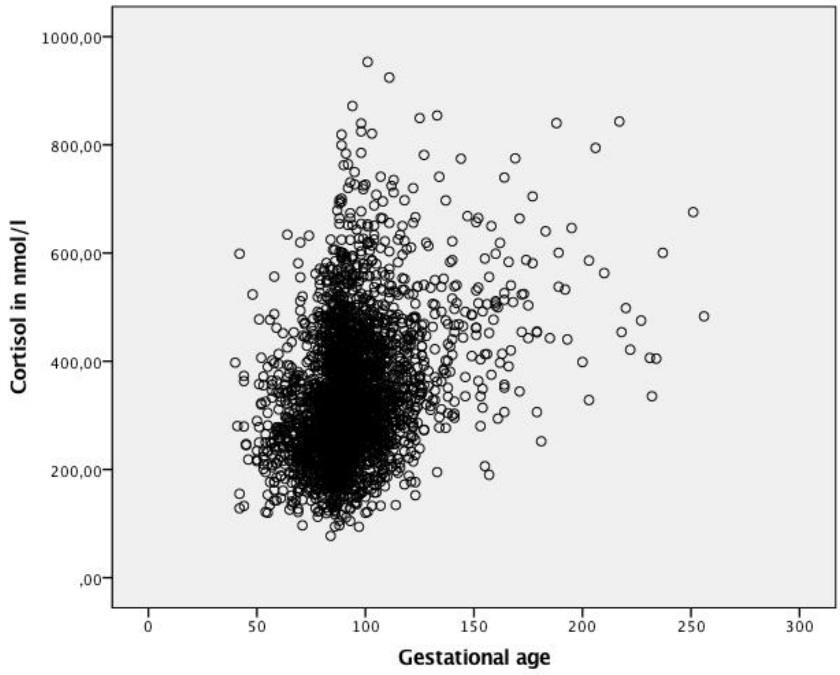

Figure 2. Scatterplot of values of plasma cortisol (nmol/l) at different times on a day (hours) (n=3039).

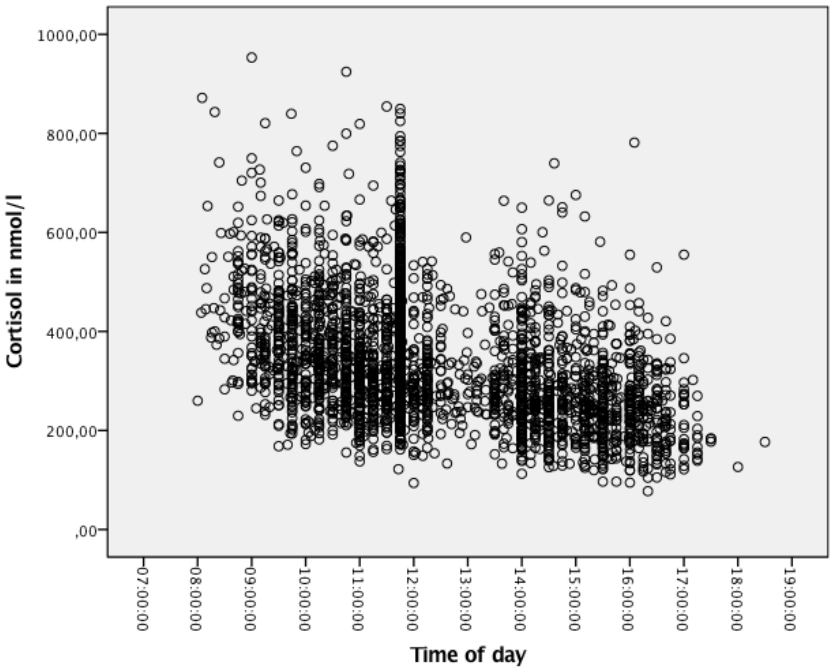

Table 1. General characteristics

	All women n= 3039	*Sensitivity analysis n= 600*	*Nulliparous women n=1749*	*Multiparous women n=1290*	*Employed women n=2387*	*Unemployed women n=630*	*Dutch women n=1935*	*Creole women n=171*	*Moroccan women n=177*	*Turkish women n=116*	*Other ethnicities n=633*
Cortisol (nmol/l)	337 (124)	386 (124)	345 (130)	320 (110)	328 (121)	356 (130)	328 (116)	350 (127)	361 (146)	348 (127)	339 (127)
Gestational age (days)	94 (21)	107 (16)	93 (21)	96 (22)	92 (18)	103 (31)	91 (17)	104 (28)	109 (31)	102 (22)	95 (25)
Time of day[1]	5.0 (2.2)	4.0 (0.0)	5.1 (2.3)	4.8 (2.1)	5.0 (2.3)	4.9 (2.0)	4.9 (2.3)	5.1 (2.2)	4.8 (2.0)	5.3 (2.0)	5.0 (2.2)
Sex of the child (boys)	48.5	50.0	49.4	47.3	48.6	48.7	48.5	44.1	49.2	52.6	48.5
Pre-pregnancy BMI[2] (kg/m^2)	22.8 (3.7)	22.8 (3.8)	22.4 (3.4)	23.3 (4.0)	22.6 (3.4)	23.5 (4.4)	22.5 (3.3)	23.9 (5.0)	25.1 (4.3)	24.0 (4.4)	22.5 (3.4)
Maternal age (years)	31.3 (4.7)	31.2 (5.3)	30.2 (4.6)	32.7 (4.3)	31.8 (4.1)	29.2 (5.9)	32.1 (3.9)	29.0 (6.3)	27.3 (5.4)	25.9 (5.1)	31.3 (4.6)
Parity											
0	57.6	55.0	100	0	62.1	39.8	59.0	56.7	45.2	44.8	59.2
1	32.1	33.8	0	75.7	31.0	36.7	33.5	28.7	26.6	32.8	30.6
≥2	10.3	11.2	0	24.3	6.9	23.5	7.1	14.6	28.2	22.4	10.2
Ethnicity											
- Dutch	63.8	62.9	65.4	61.7	72.8	29.1	-	-	-	-	-
- Creole	5.6	5.3	5.6	5.7	4.5	10.2	-	-	-	-	-
- Turkish	3.8	4.0	3.0	5.0	1.8	11.8	-	-	-	-	-
- Moroccan	5.8	7.8	4.6	7.5	2.9	17.1	-	-	-	-	-
- Other	20.9	19.9	21.5	20.0	18.0	31.8	-	-	-	-	-
Smoking	8.3	8.8	8.3	8.3	7.5	11.0	8.5	14.6	2.8	19.0	5.5
Alcohol consumption	26.8	28.3	24.6	29.8	30.3	13.5	32.5	11.7	0.6	0.9	25.8
Years of post-primary education	9.6 (3.7)	9.7 (3.8)	9.9 (3.4)	9.1 (4.0)	10.4 (3.1)	6.7 (4.3)	10.6 (2.9)	8.2 (4.2)	5.0 (3.5)	4.9 (3.6)	9.1 (4.0)
Paid job	79.1	77.6	85.5	70.5	100	0	90.5	62.6	39.2	36.2	68.4
Pre-existing disease	19.6	20.2	19.6	19.5	18.8	22.5	18.6	22.8	20.3	26.7	20.2

Pre-pregnancy medication	11.0	11.3	11.9	9.7	10.6	12.9	11.1	9.9	10.2	6.9	12.0
Disease during pregnancy	26.5	25.1	24.8	28.9	25.1	31.9	23.7	35.6	37.3	33.6	28.4
- STD[3]	1.8	2.2	1.6	2.0	1.5	3.0	1.5	4.8	3.0	1.8	1.6
- Gestational diabetes	0.5	0.2	0.2	0.8	0.3	1.1	0.3	0.6	1.8	0.0	0.8
- PIH[4]	2.0	0.5	1.6	2.4	1.6	3.4	1.6	3.0	3.6	2.7	2.6
- Vaginal blood loss	11.0	10.9	10.8	11.2	10.9	11.1	10.4	13.4	11.8	11.5	11.7
- Anemia	4.0	3.0	3.5	4.7	3.5	6.0	2.5	8.6	13.2	4.4	4.8
- Rubella	1.4	0.0	0.2	0.1	0.0	0.7	0.0	0.0	1.2	0.9	0.2
- Other	12.8	12.6	12.0	14.0	12.2	15.1	11.7	15.8	15.3	21.5	13.5
- Nausea/vomiting	46.5	44.8	43.0	51.2	44.0	56.0	42.7	55.0	59.9	66.4	48.5
- >5kg weight loss	4.5	4.2	4.5	4.5	3.1	9.8	2.7	8.8	14.7	12.1	4.6
Medication use during	35.6	38.3	35.4	36.0	34.3	41.3	34.9	42.7	32.8	44.8	35.2
- Antibiotics	3.8	3.3	3.5	4.0	3.3	5.4	2.9	8.2	4.0	7.8	4.3
- Analgesics	22.6	26.5	21.8	23.7	22.1	24.6	23.3	21.6	19.2	26.7	21.0
- Antihypertensives	0.4	0.0	0.3	0.5	0.5	0.2	0.3	2.3	0.0	0.0	0.5
- Anti-emetics	4.4	3.8	3.1	6.1	3.4	8.1	2.8	11.1	7.3	15.5	4.4
- NSAID's[5]	0.5	0.0	0.5	0.5	0.5	0.5	0.5	1.8	99.4	0.9	0.3
- Anti-epileptics	0.1	0.0	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.2
- Thyroid hormones	0.6	0.8	0.5	0.7	0.7	0.2	0.6	0.0	0.0	0.9	0.6
- Thyroid inhibitors	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.2
- Benzodiazepines	0.5	0.3	0.6	0.4	0.4	1.1	0.6	0.6	0.0	1.7	0.3
- Insulin	0.1	0.0	0.0	0.2	0.1	0.0	0.2	0.0	0.0	0.0	0.0
- Other	11.4	12.7	12.5	10.0	10.9	13.7	11.5	11.1	9.6	6.9	12.8
CRP[6](mg/l)	5.1 (6.9)	5.8 (7.9)	4.2 (5.4)	6.3 (8.4)	4.9 (7.0)	5.9 (6.6)	4.9 (6.6)	5.6 (6.2)	7.3 (7.6)	6.1 (6.1)	4.9 (7.9)
Multidimensional Fatigue	2.4 (0.5)	2.4 (0.5)	2.4 (0.5)	2.5 (0.5)	2.4 (0.5)	2.5 (0.5)	2.4 (0.5)	2.5 (0.6)	2.6 (0.5)	2.7 (0.5)	2.4 (0.5)
Exercise	89.8	91.0	90.5	88.8	91.8	82.1	94.8	70.8	70.6	68.1	88.9
PRAQ[7] Score	20.6 (4.7)	20.8 (4.8)	21.8 (4.6)	18.9 (4.4)	20.4 (4.5)	21.1 (5.5)	20.1 (4.3)	20.4 (5.4)	21.4 (5.6)	23.5 (5.7)	21.2 (4.9)
Job Demand	53.0 (9.6)	52.9 (9.9)	53.3 (8.9)	52.7 (10.7)	53.7 (8.4)	-	53.6 (8.1)	51.6 (11.0)	48.0 (17.2)	54.0 (15.9)	51.4 (11.5)
Job Control	31.9 (6.9)	31.9 (7.2)	32.0 (6.8)	31.8 (7.0)	32.1 (6.7)	-	32.3 (6.6)	31.6 (7.6)	29.6 (8.1)	27.6 (8.3)	31.5 (7.1)
Parenting Daily Hassles	34.8 (8.7)	35.3 (8.5)	-	35.6 (7.7)	34.3 (8.1)	36.2 (9.8)	34.3 (7.8)	32.8 (10.1)	35.9 (9.9)	37.8 (9.2)	35.9 (10.3)
CES-D[8]	31.5 (8.1)	31.5 (8.1)	31.3 (7.9)	31.9 (8.6)	30.8 (7.5)	34.4 (9.8)	30.4 (7.3)	34.2 (10.4)	34.5 (10.7)	36.4 (9.9)	32.3 (8.2)

STAI[9]	36.8 (10.0)	37.3 (10.1)	36.5 (9.8)	37.2 (10.3)	35.9 (9.5)	40.4 (11.1)	35.2 (9.3)	40.3 (12.0)	41.3 (11.2)	43.8 (9.9)	38.8 (10.3)

Notes: For binary and categorical variables (i.e. sex and parity), numbers represent percentage. For continuous variables (i.e. cortisol, age), numbers represent means, and numbers inside parentheses represent standard deviations. [1]= Continuous variable, coded as '1' for samples taken between 08:00 and 09:00 am, as '2' for samples taken between 09:00 and 10:00 am, and so on, with a maximum of '11' for samples taken after 06:00 pm;[2]= Body mass index; [3]= Sexually transmitted disease; [4]= Pregnancy-induced hypertension; [5]= Non-steroid anti-inflammatory drugs; [6]= C-reactive protein; [7]= Pregnancy Related Anxiety Questionnaire; [8] = Center for Epidemiologic Studies Depression scale; [9]= State-Trait Anxiety Inventory

Table 2. Regression analysis showing all the independent variables contributing to the variance in serum cortisol levels (nmol/l) in all women.

	Univariate (n=3039)		Multivariate[1] (n=3039)		Multivariate[2] (n=2974)	
Biological	**B**	**95%-CI**	**B**	**95%-CI**	**B**	**95%-CI**
Maternal age (years)	**-3.6**	**-4.6 to -2.7†**	**-2.7**	**-3.5 to -1.9†**	**-1.5**	**-2.4 to -0.6****
Multiparous	**-15.8**	**-21.4 to -10.3†**	**-23.5**	**-28.2 to -18.9†**	**-21.5**	**-27.1 to -15.9†**
Pre-pregnancy BMI (kg/m^2)	**-1.0**	**-2.2 to 0.1***	**-1.9**	**-2.9 to -0.9†**	**-1.3**	**-2.4 to -0.3****
CRP (mg/l)	**1.1**	**0.5 to 1.8†**	**0.5**	**-0.1 to 1.0***	**1.0**	**0.4 to 1.5†**
Disease before pregnancy	1.7	-9.3 to 12.7	-0.2	-9.5 to 9.2		
Disease during pregnancy	6.3	-3.7 to 16.2	3.5	-5.0 to 11.9		
Nausea/vomiting	0.3	-8.5 to 9.1	0.0	-7.4 to 7.4		
>5kg weight loss during pregnancy	**21.2**	**0.2 to 42.3****	3.6	-14.3 to 21.5		
Dutch	-15.3	-24.4 to -6.2	-3.1	-10.9 to 4.8	12.8	-63.4 to 89.0
Creole	17.5	-1.4 to 36.5	2.9	-13.3 to 19.1	16.1	-61.3 to 93.6
Turkish	13.6	-9.2 to 36.4	6.1	-13.3 to 25.5	11.8	-66.5 to 90.0
Moroccan	**26.7**	**8.1 to 45.4****	-4.3	-20.4 to 11.7	8.9	-68.6 to 86.3
Other	4.0	-6.8 to 14.8	3.5	-5.6 to 12.6	13.2	-63.1 to 89.5
Female fetus	**9.6**	**0.9 to 18.3****	**8.4**	**1.0 to 15.8****	**9.2**	**1.8 to 16.5****
Lifestyle						
Alcohol	**-8.7**	**-18.6 to 1.2***	-5.2	-13.5 to 3.2		
Smoking	-11.1	-27.0 to 4.7	**-13.2**	**-26.6 to 0.2***	**-14.2**	**-27.7 to -0.6****
Medication before pregnancy	1.2	-12.7 to 15.2	0.7	-11.1 to 12.5		
Medication during pregnancy	1.1	-8.0 to 10.3	-2.1	-9.8 to 5.6		
Exercising	-6.2	-20.6 to 8.2	-3.2	-15.4 to 9.1		
Fatigue	**-9.1**	**-17.9 to -0.3****	-2.7	-10.1 to 4.7		
Insufficient sleep	**-12.5**	**-21.4 to -3.6****	**-11.7**	**-19.3 to -4.2****	**-8.5**	**-16.1 to -0.9****
Years of education	**-1.4**	**-2.5 to -0.2****	0.8	-0.2 to 1.8		
Employed	**-27.1**	**-37.8 to -16.3†**	-5.3	-14.6 to 4.1	**-12.7**	**-23.2 to -2.2****
Stress						
PRAQ	6.3	-3.1 to 15.6	**10.8**	**2.9 to 18.8****	2.3	-6.2 to 10.8
Job Demand	-4.7	-18.5 to 9.1	2.5	-9.2 to 14.1		

Job Control	-2.6	-10.5 to 5.3	-2.7	-9.5 to 4.0
Parenting Hassles	9.8	-5.2 to 24.8	2.2	-10.6 to 15.0
Depression	8.1	-2.5 to 18.7	7.1	-1.9 to 16.1
Anxiety	6.7	-2.0 to 15.4	4.1	-3.3 to 11.5

**=p<0.1, **=p<0.05, †=p<0.001. B=regression coefficient. 95%-CI = 95%-Confidence Interval. [1]= adjusted for gestational age and time of day. [2]= adjusted for gestational age, time of day, maternal age, parity, pre-pregnancy BMI, CRP, smoking, sleeping sufficiency, Pregnancy Related Anxiety, employment status and ethnicity.*

R^2= 0.3

Table 3. Regression analysis showing all the independent variables contributing to the variance in serum cortisol levels (nmol/l) in different subgroups.

	All **n=2974**	**Nulliparous** **n=1715**	**Multiparous** **n=1258**	**Employed** **n=2196**	**Unemployed** **n=613**	**Dutch** **n=1901**	**Creole** **n=165**	**Turkish** **n=114**	**Moroccan** **n=165**	**Other** **n=618**	**Sensitivity** **n=582**
Biological	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
Maternal age (years)	**-1.5****	**-1.9****	-0.5	**-1.4****	**-1.7****	**-1.5****	**-3.6****	-0.3	0.6	-1.9	-0.9
Multiparous	**-21.5†**	-	**-14.7****	**-21.5†**	**-22.8†**	**-20.4†**	-6.7	**-55.9†**	**-38.7†**	**-14.9****	**-31.0†**
Pre-pregnancy BMI (kg/m^2)	**-1.3****	**-2.9†**	0.5	**-2.3****	0.9	**-1.7****	-2.7	2.4	0.5	-0.5	**-3.0****
CRP (mg/l)	**1.0†**	**1.3****	**0.9****	**0.9****	0.7	**1.0****	1.5	-2.6	**2.4****	0.7	0.0
Female fetus	**9.2****	**11.9****	6.9	**12.5****	6.2	2.7	9.7	**34.6***	-10.3	**29.5****	15.1
Lifestyle											
Smoking	**-14.2****	**-22.3****	-2.2	**-14.8***	-13.5	**-20.3****	-29.6	17.6	-25.5	6.1	10.7
Insufficient sleep	**-8.5****	-8.0	-6.7	-4.5	**-18.5****	-4.7	12.4	-30.4	**-35.6***	**-20.0****	-5.5
Employed	**-12.7****	-16.1	-6.7	-	-	-11.2	2.4	**-47.7****	-24.2	-6.5	0.1
Stress											
PRAQ	2.3	6.1	**-12.0***	-0.7	10.3	2.7	-14.5	4.8	7.3	8.3	-8.6
Job Demand				0.9							
Job Control				-2.4							
Parenting Hassles			8.7								

**=p<0.1, **=p<0.05, †=p<0.001. B=regression coefficient. All results are adjusted for gestational age, time of day, maternal age, parity, pre-pregnancy BMI, CRP, smoking, sleeping sufficiency,*

Pregnancy Related Anxiety employment status and ethnicity. Job hassles were included only in employed women and parenting hassles only in multiparous wo