Maternal chronic stress correlates with serum levels of cortisol, glucose and C-peptide in the fetus, and maternal acute stress with fetal growth

Georgios Valsamakis [a,b,c], Dimitrios Papatheodorou[a], Nikolaos Chalarakis N[a], Maria Manolikaki[a], Alexandra Margeli[d], Ioannis Papassotiriou I[d], Thomas M Barber[c], Sudhesh Kumar[c], Sophia Kalantaridou[b], George Mastorakos[a]

[a]Endocrine Unit, 2nd Department of Obstetrics and Gynecology, Aretaieion University Hospital, Athens Medical School, Athens, Greece

[b]3rd Department of Obstetrics and Gynecology, Attikon University Hospital, Athens Medical School, Athens, Greece

[c]Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM), University Hospital Coventry and Warwick Medical School, Coventry, UK

[d]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece

Running Title: acute and chronic stress, fetal growth and metabolism

Corresponding author:

George Valsamakis

Assistant Professor Endocrinology

37A Leoforos Pendelis Avenue

Vrilissia/Athens

15235 Greece

keywords: chronic stress, fetal cortisol, c-peptide, waist

Highlights

- **Pregnancy stressors associate with changes in maternal and fetal HPA axes**
- **Maternal acute stress impacts on fetal growth, with increased fetal abdominal growth and reduced fetal head growth**
- **Maternal chronic stress increases fetal cortisol, glucose and c-peptide secretion**

Abstract

Introduction: During pregnancy, maternal stressors cause changes in both maternal and fetal HPA axes. We therefore investigated the impact of maternal acute and chronic stress on fetal glucose metabolism and growth, and serum levels of cortisol in the fetus.

Materials and Methods: Normal weight pregnant women (n=192; mean±SD 27.9±4.2 years old, and; 26.9±2.4 kg/m^2) were assessed during the 2nd and 3rd trimester with anthropometry, fetal ultrasound, blood samples for serum CRH, cortisol and IL6, and STAI trait and state stress questionnaires. We measured serum cortisol, insulin and c-peptide, and plasma glucose from cord blood. Neonates underwent anthropometry at the 3rd post-delivery day.

Results: In both 2nd and 3rd trimesters, women with STAI trait scores ≥40 had significantly greater levels of fasting serum CRH and cortisol than those with STAI trait scores<40.

2nd trimester: STAI trait scores correlated positively with cord blood glucose and c-peptide. Maternal serum CRH correlated negatively with U/S fetal biparietal head diameter, while serum cortisol correlated positively with abdominal circumference. Maternal serum IL6, CRH and cortisol all correlated positively with birth waist circumference.

3rd trimester: Women with STAI state scores ≥40 had fetuses with larger U/S abdominal and smaller head circumferences compared to those of women with STAI scores <40. Women with STAI trait scores ≥40 had greater levels of cord blood cortisol, glucose, and c-peptide compared to women with STAI scores <40. STAI state scores ≥40 correlated positively with maternal CRH and U/S fetal abdominal circumference, and negatively with fetal head circumference and biparietal diameter. STAI trait scores correlated positively with cord blood c-peptide, glucose, insulin and cortisol. Maternal serum levels of CRH correlated positively with U/S fetal abdominal circumference and cord blood cortisol, and negatively with fetal head circumference and biparietal head diameter. Maternal serum levels of both CRH and cortisol correlated positively with cord blood c-peptide, glucose, and insulin. STAI trait was the best positive predictor of cord blood cortisol, glucose and c-peptide, whilst STAI state was

the best positive and negative predictor, respectively of fetal abdominal circumference and fetal head circumference or biparietal diameter.

Conclusions: Increased maternal chronic stress (reflected by the STAI trait score) associates with increased fetal cortisol, glucose, c-peptide secretion and thus, insulin resistance. Maternal acute stress (STAI state) in the 3rd trimester associates with changes in fetal growth pattern, including increased and decreased measurements of fetal abdominal and head growth respectively**.**

1. Introduction

‘‘Stress’’ is a state of disharmony or threat to homeostasis. The hypothalamic-pituitary-adrenal (HPA) axis and the locus ceruleus–norepinephrine (LC/NE) autonomic systems are the main components of the endocrine response to stress (Croussos & Gold ,1992). During pregnancy, a state of relative hypercortisolism ensues, with increased serum levels of CRH (secreted from the placenta) in the maternal circulation, especially during the 3rd trimester (Magiakou et al., 1996). Placental CRH has been proposed as a potential predictor for preterm, term, or post-term labor (Mastorakos & Ilias, 2000). During pregnancy, chronic or acute maternal stressors may influence the function of both maternal and fetal HPA axes in the short-term, and offspring physiology in the long-term. In sheep, chronic psychosocial maternal stress during early gestation associates with increased levels of serum cortisol (both baseline and stress-induced) and norepinephrine in the fetus, indicating hyperactivity in the fetal HPA-axis and sympathetic-adrenal-medullary system (Bischoff et al., 2018). Premature activation of the fetal HPA axis may result from an adverse intra-uterine environment, such as hypoxemia. Excessive serum levels of glucocorticoids in the fetus due to sustained endogenous fetal cortisol production, associates with intrauterine growth restriction (Challis et al., 2001).

Insulin resistance is a feature of normal pregnancy, described as an adaptive phenomenon to divert maternal glucose towards fetal needs (Radaelli et al., 2003). We have shown previously in a cohort of normal pregnant women, that long-term stress (assessed through use of the STAI trait questionnaire) associates with decreased maternal insulin sensitivity (Valsamakis et al., 2017). Other studies in humans have shown that exposure of the mother to prenatal psychosocial stress associates with development of insulin resistance in the offspring, assessed during young adulthood (Etringer et al., 2008). Similar studies in rats have shown that maternal exposure to prenatal stress associates with induction of intrauterine growth restriction (IUGR) and glucose intolerance (Lesage et al., 2004). Furthermore, chronic heat stress in maternal ewes induced a shift towards glucose metabolism within the fetal liver, which as the authors stated, could be explained by stress- and cortisol-induced increased

activity of gluconeogenic enzymes within the fetal liver (Dreilling et al., 1991).

Low birth weight (LBW), prematurity and IUGR remain leading causes of perinatal morbidity, mortality, neurodevelopmental impairments and disabilities among newborn babies (WHO, 1995). Placental CRH, cortisol and other hormones that cross the placenta, could result in retardation of fetal growth rate, reduced birth weight and precipitation of preterm labor in prenatally stressed fetuses (Weinstock, 2005). Animal studies confirmed that increased serum levels of stress hormones and pro-inflammatory cytokines in the fetal compartment during sensitive or critical developmental windows, can have an impact on the structure and function of the brain and peripheral targets that are related to body composition, energy balance homeostasis and metabolic function (i.e., adipose tissue, pancreas, and liver) (Raternain, 2013; Lesage, 2004). Current evidence suggests that maternal stress experienced early in gestation, has more serious consequences for the offspring than stress experienced later in gestation (Mueller & Bale, 2007).

STAI is a well-standardized, self-reported questionnaire designed to measure both ‘state’ and ‘trait’ anxiety. Feelings of anxiety may occur in stressful situations. State anxiety refers to fear, nervousness, discomfort, and arousal of the autonomic nervous system, all induced temporarily by situations perceived as dangerous (i.e., how a person is feeling at the time of a perceived threat). Conversely, trait anxiety refers to a relatively enduring predisposition to feelings of stress, worry, and discomfort (Sielberger & Sydeman, 1994). Within the literature, there is good establishment and usage of the STAI trait and state questionnaire for use in pregnancy for the purpose of assessment of maternal stress (Rondo et al., 2003; Di Pietro et al., 2006). However, to date there is insufficient data regarding the impact of duration of maternal stress (acute *vs* long-term) on fetal growth and/or metabolism. To address this, we investigated the impact of maternal acute and chronic stress (evaluated by STAI state and trait questionnaires and maternal serum levels of CRH, cortisol, and IL6) on serum levels of cortisol, glucose metabolism and growth pattern within the fetus.

2. Materials and Methods

2.1. Subjects

Primigravidae caucasian women (n=220) were recruited during the first trimester of pregnancy from an antenatal outpatient clinic of a university hospital in Greece, between May 2015 and December 2017. To avoid bias, we used a computer software random numbers generator for recruitment purposes. Women who presented with miscarriages during the first trimester of pregnancy (n=12) were not included in the protocol. Following psychiatric assessment during the first trimester, women with past or current severe mental health problems such as severe depression or anxiety (n=8) were excluded from the study. Accordingly, n=200 women were included in the study protocol (age, mean±SD: 28.2±4.5 years; pre-pregnancy BMI: 27.1±2.6 kg/m^2). Following recruitment, initiation of execution of the study occurred during the 2nd trimester of pregnancy. Exclusion criteria included:

- Non-caucasian origin (to maintain population homogeneity regarding insulin resistance traits)
- Pre-pregnancy BMI>30 kg/m^2
- History of type 1 or type 2 diabetes mellitus or glucose intolerance
- Presence of gestational diabetes (GDM)
- Multiple pregnancy
- Major vaginal bleeding
- Hypertension
- Preeclampsia
- Urinary tract infection
- Fetal-placental abnormalities (such as congenital anomalies)
- Placenta previa and/or placental abruption
- Nephropathy and/or liver disease
- Current smoking and/or alcohol intake.

The study was approved by an institutional ethical committee, functioning according to the 4th edition of the Guidelines on the Practice of Ethical Committees in Medical Research, issued by the Royal College of Physicians of London (Royal College of Physicians, 2007). We obtained full informed consent from each recruited pregnant woman, following detailed explanation of the purpose and nature of the research protocol**.**

2.2. Protocol

Recruited women were seen once during each of the 2nd and 3rd trimesters of their pregnancy between 24 th-26 th and 34 th-36 th week of gestation, respectively. Recruited women received basic dietetic advice at their initial visit, without regular dietetic follow up. At each study visit, recruited women underwent anthropometric measurements that included fasting blood sampling at 8 am (assessment of serum CRH, cortisol and IL6) and a 75-gram oral glucose tolerance test (OGTT), with blood samples drawn at 0, 30, 60, 90 and 120 min time-points for assessment of plasma glucose and insulin concentrations. At each study visit, recruited women also had a pelvic ultrasound assessment and self-completed a STAI trait and state questionnaire. Application of diagnostic criteria set by WHO (based on OGTT) for diagnosis of GDM (World Health Organization, 2013**)**, resulted in exclusion of n=8 women from the study. The study cohort therefore had n=192 pregnant women (age and pre-pregnancy BMI: 27.9±4.2 years and 26.9±2.4 kg/m², respectively, Table 1**).** Neonates underwent measurements of birth weight at birth, and waist circumference on the 3rd post-partum day. We collected all blood (including cord blood) samples for measurement of hormones (cortisol, c peptide and insulin) and glucose in EDTA tubes. We added millipore's serine protease inhibitor to each blood sample following its collection, followed by multiple tube inversion and immediate centrifugation. Following centrifugation, we collected plasma from each sample, and stored these as aliquots at –70°C.

2.3. Stress Questionnaires

STAI trait and state self-rate questionnaires are composed of 20 questions each. The former provides data on trait characteristics of the subject, including how the individual

usually feels. The latter provides data on how the subject feels at the specific time of completion of the questionnaire.

2.4. Anthropometrics and blood pressure measurements

A single observer executed anthropometric assessments of pregnant women. At pre-pregnancy and at each study visit, we measured weight and height (the former without shoes and light clothing in kilograms to the nearest 0.1 kg on a beam balance; the latter to the nearest mm using a stadiometer). BMI in kg/m^2 was calculated. Blood pressure was measured twice (mean value calculated) following sitting for 10 minutes and a 5-minute duration between measurements. We measured birthweight in kilograms with a portable digital electronic scale (Seca GMBH and Co. kg Germany, model 834), accurate to the nearest 10 grams, without clothing or diapers. We measured neonatal waist circumference using a measuring tape at the umbilical level.

2.5. Fetal ultrasound measurements

During ultrasonography, a single observer recorded fetal measurements (estimated weight, abdominal circumference, head circumference and biparietal diameter). We employed a Philips HD11 ultrasonographer to perform all ultrasonography.

2.6. Blood chemistry and hormone assays

We measured all analytes in maternal sera and cord blood using the same methodology for both types of samples. We measured plasma glucose concentrations using the Roche Cobas 6000 Clinical Chemistry System (Roche Diagnostics Rotkreuz, CH). We measured serum insulin, cortisol and C-peptide concentrations with electrochemiluminescence immunoassays, using the Roche Cobas e411 immunochemistry analyzer (Roche Diagnostics Rotkreuz, CH). Serum IL-6 was measured by the quantitative sandwich enzyme immunoassay technique (Quantikine, R&D Systems, Minneapolis, MN, USA) with the intra- and inter- assay CVs ranging between 6.9 and 7.4% and between 6.5 and 9.6%, respectively. Serum CRH was measured by a quantitative kit based on the principle of "competitive" enzyme immunoassay (Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA) with intra- and inter- assay CVs at <10% and <12%, respectively, according to the

manufacturer.

2.7. Statistical analysis

We present data as mean ± SD or median and interquartile range (25^{th}-75^{th} percentile) for data normally and non-normally distributed, respectively. To analyse the change of each variable during pregnancy, we used the one-way repeated measures ANOVA test for normally distributed variables, and the non-parametric Friedman ANOVA test for non-normally distributed variables. For assessment of correlations between different variables, we performed Spearman correlation analyses. Stepwise multiple regression analysis was used to define 2^{nd} and 3^{rd} trimester predictive variables. A *p-value* of <0.05 was considered to be significant. We used the SPSS statistical software for statistical analysis (SPSS Inc., 1999).

3. Results

3.1. Anthropometric, hormonal, metabolic and psychometric variables

Maternal weight increased significantly between each trimester ($p<0.05$), with a median increase of 11.5 kg between the 1st and 3rd trimester. Maternal systolic blood pressure values increased significantly from the 2nd to the 3rd trimester. Maternal fasting serum cortisol and CRH concentrations increased significantly from the 2nd to the 3rd trimester, whilst serum IL6 concentrations did not. STAI state scores showed a statistically significant increase from the 2nd to the 3rd trimester, whilst STAI trait scores remained static. Ultrasound-based fetal measurements of estimated weight, abdominal circumference, head circumference and biparietal diameter increased significantly from the 2nd to the 3rd trimester. Neonatal birth weight values were within normalcy (Table 1).

In the 2nd and 3rd trimesters, women with STAI trait scores ≥40 had significantly greater serum fasting CRH levels (2.3 ±0.3 ng/ml and 2.8±0.2 ng/ml respectively), than those with STAI trait scores <40 (2.1±0.4 ng/ml and 2.6±0.5 ng/ml respectively; $p<0.001$ and $p<0.001$ respectively). In the 2nd and 3rd trimesters, women with STAI trait scores ≥40 had significantly greater fasting serum cortisol levels (25±7.2 μg/dL and 31±7.9 μg/dL respectively), than those with STAI trait scores <40 (20±7.6 μg/dL and 26±7.3 μg/dL respectively; $p<0.001$ and $p<0.001$ respectively).

In the 3rd trimester, fetuses of women with STAI state scores ≥40 had larger abdominal and smaller head circumferences compared to those of women with STAI state scores <40. There was no difference in neonatal birth weight in these two groups of women (Table 2).

In the 3rd trimester, women with STAI trait scores ≥40 had significantly greater levels of cord blood cortisol, glucose and c-peptide compared to women with STAI trait scores <40 (Table 3).

3.2. **Correlations of maternal serum IL6, CRH and cortisol concentrations with fetal ultrasound measurements, birth waist circumference, and cord blood glucose, insulin, c-peptide and cortisol concentrations**

2nd trimester: Maternal serum CRH concentrations correlated positively with maternal serum cortisol concentrations (p=0.038, r=0.328), and negatively with ultrasound-measured fetal biparietal head diameter (p=0.039, r=-0.357). Maternal serum cortisol concentrations correlated positively with ultrasound measured fetal abdominal circumference (p=0.030, r=0.823). Maternal serum IL6, CRH and cortisol concentrations all correlated positively with birth waist circumference (p=0.038, r=0.527; p=0.032, r=0.800 and p=0.034, r=0.790 respectively. Table 4|).

3rd trimester: Maternal serum CRH concentrations correlated positively with maternal serum cortisol concentrations (p=0.042, r=0.301), ultrasound-measured fetal abdominal circumference (p=0.031, r=0.396) and cord blood cortisol concentrations (p=0.029, r=0.589). Maternal serum IL6 and CRH concentrations both correlated negatively with fetal head circumference (p=0.001, r=-0.865; p=0.033, r=-0.385 respectively), and fetal biparietal head diameter measurements (p=0.013, r=-0.695; p=0.032, r=-0.355 respectively). Maternal serum CRH and cortisol concentrations both correlated positively with levels of cord blood c-peptide (p=0.022, r=0.684; p=0.026, r=0.614 respectively), glucose (p=0.027, r=0.655; p=0.035, r=0.515 respectively. Figure 1), and insulin (p=0.024, r=0.679; p=0.023, r=0.586 respectively. Table 5).

3.3. **Correlations of maternal psychometric variables (STAI state and trait questionnaires) with fetal ultrasound measurements and cord blood glucose, insulin, c-peptide and cortisol concentrations**

2nd trimester: Maternal STAI state scores correlated positively with maternal CRH concentrations (p=0.044, r=0.455). STAI trait scores correlated positively with cord blood glucose (p=0.042, r=0.618) and c-peptide (p=0.041, r=0.589) concentrations (Table 4).

3rd trimester: Maternal STAI state scores ≥40 correlated positively with maternal CRH concentrations (p=0.020, r=0.817) and ultrasound measured fetal abdominal circumference (p=0.024, r=0.548), and negatively with measurements of fetal head circumference (p=0.027, r=-0.362) and fetal biparietal diameter (p=0.009, r=-0.534). Maternal STAI trait scores correlated positively with levels of cord blood c-peptide (p=0.032, r=0.524), glucose (p=0.035, r=0.512), insulin (p=0.033, r=0.517) and cortisol (p=0.035, r=0.505. Table 5).

3.4. Predictors of fetal hormonal profile and growth patterns

In the 2nd trimester, multiple regression analysis showed that maternal serum cortisol concentrations were the best positive predictors of ultrasound measured fetal abdominal circumference (p=0.018, beta=0.782), with maternal serum levels of IL6, CRH and cortisol, maternal weight and maternal STAI state and trait scores all taken as independent variables.

In the 3rd trimester, multiple regression analysis showed that maternal STAI trait scores were the best positive predictors of levels of cord blood cortisol (p=0.035, beta=0.678), glucose (p=0.039, beta=0.601) and c-peptide (p=0.003, beta=1.085), with maternal serum IL6, CRH and cortisol concentrations, maternal weight, and STAI state and trait scores all taken as independent variables.

In the 3rd trimester, multiple regression analysis showed that maternal STAI state scores were the best positive predictors of ultrasound measured fetal abdominal circumference (p=0.021, beta=0.778). Maternal STAI state scores were also the best negative predictors of ultrasound measured fetal head circumference (p=0.032, beta=-0.723) and biparietal diameter (p=-0.009, beta=-0.965), with maternal serum IL6, CRH and cortisol concentrations, maternal weight, and STAI state and trait scores all taken as independent variables.

4. **Discussion**

We demonstrate that in normal weight pregnant women, maternal STAI state scores and fasting serum levels of CRH and cortisol concentrations increase from the 2nd to the 3rd trimester. In the 2nd trimester, maternal serum CRH concentrations correlate positively with maternal STAI state scores and maternal serum cortisol concentrations, whilst in the 3rd trimester, maternal serum CRH concentrations correlate positively with maternal STAI state scores ≥40 and maternal serum cortisol concentrations. These findings are in accordance with previously published findings (Valsamakis G et al., 2017). Levels of placental CRH and maternal serum cortisol increase during pregnancy, and reflect maternal acute stress as scored subjectively by STAI state questionnaire. Stress-related stimulation of the maternal sympathetic nervous system can lead to enhanced placental CRH secretion via a reduction of uterine blood flow and placental hypo-perfusion (Texeira et al., 1979; Jones et al., 1989). We also demonstrate that in the 3rd trimester, women with STAI trait scores ≥40 have greater levels of serum cortisol and cord blood cortisol, compared to women with STAI trait scores <40. This suggests that maternal chronic stress (as reflected by higher STAI trait scores) associates with increased levels of cortisol both within the maternal and fetal circulations. We confirmed these observations with 3rd trimester STAI trait scores as the best positive predictor of levels of cord blood cortisol, and positive correlations between 3rd trimester STAI trait scores and placenta-derived CRH concentrations with levels of cord blood cortisol.

In a recently reported study of normal pregnancies in the 3rd trimester, maternal pregnancy-specific stress assessed by the Prenatal Distress Questionnaire (PDQ, a 12-item scale), had a positive association with levels of neonatal hair cortisol, a marker of fetal cortisol concentrations during pregnancy (Romero-Gonzalez et al., 2018). During pregnancy, stress-induced increased levels of maternal serum cortisol may cross the placenta by overwhelming the protective placental barrier of 11βHSD2 action (Rakers et al., 2017; Konstantakou et al., 2017). In contrast to the inhibitory effect of cortisol on hypothalamic CRH production, maternal cortisol *stimulates* placental CRH production. This positive feedback loop, in turn stimulates the fetal HPA axis resulting in increased levels of fetal

cortisol (Glover et al., 2009). Thus, chronic maternal stress and elevated levels of placental CRH result in increased levels of neonatal cortisol, and may induce epigenetic changes in the offspring's HPA function. However, in one study on pregnancy outcomes following the effects of acute stress experienced during the 9/11 events, offspring had *reduced* levels of serum cortisol (Yehuda et al., 2005). Thus, re-programming effects of maternal stress on the offspring's HPA axis may manifest differential translation depending on the amplitude and type of stressor, and the timing of exposure to it as previously suggested, resulting in either increased or decreased baseline cortisol production in the offspring (Romero-Gonzalez et al., 2018). Existing data imply that there could exist a transgenerational manifestation of maternal stress inheritance into the fetus, and further studies are required to examine this evidence (Glover et al., 2009; Van den Bergh et al., 2017).

In the 3rd trimester, maternal STAI trait scores, serum CRH and cortisol concentrations correlated positively with cord blood c-peptide, glucose and insulin concentrations. Furthermore, women with STAI trait scores ≥40 in the 3rd trimester showed significantly greater cord blood c-peptide and glucose concentrations compared to those with STAI trait scores <40. Thus, it appears that chronic maternal stress associates with increased cord blood glucose, c-peptide and insulin concentrations, all markers of fetal hyperinsulinemia and insulin resistance. It seems that chronic (STAI trait) and not acute (STAI state) maternal stress associates with development of fetal hyperinsulinemia. Of note, cord blood measurements reflect fetal metabolism at birth (Martin et al., 2015). Maternal stress-associated increase of fetal cortisol, glucose and insulin concentrations suggests that maternal stress relates to development of fetal insulin resistance. These results should be corroborated with a study of the offspring of Holocaust survivors and controls of non-exposed mothers, concluding that the former showed a higher risk for having two or more metabolic syndrome conditions (e.g., hypertension, dyslipidemia, type 2 diabetes, and overweight) compared to the latter (Flory et al., 2011). In addition, in a large epidemiological study, maternal bereavement from death of someone close during pregnancy was shown to associate with an increased risk of overweight and type 2 diabetes in offspring during later childhood

(Li et al., 2010; Dreilling et al., 2018; Brunton et al., 2013). Furthermore, maternal prenatal stress associates with stress-induced hyperglycemia in male but not female rat offspring, whilst hyperinsulinemia was noted in response to glucose loading in female but not male offspring, implying a sexually-dimorphic effect in rat offspring in response to maternal stress (Brunton et al., 2013; Qian et al., 2015).

In our study, we demonstrate that during the 2nd trimester, maternal serum CRH and cortisol concentrations correlate negatively and positively with ultrasound measurements of fetal biparietal diameter and abdominal circumference respectively. Also in the 2nd trimester, maternal cortisol concentrations were the best positive predictors of ultrasound measured fetal abdominal circumference amongst maternal serum levels of IL6, CRH and cortisol, maternal weight and STAI state and trait scores. Maternal serum IL6, CRH, and cortisol concentrations correlated positively with birth waist circumference. In the 3rd trimester, maternal STAI state scores, serum CRH and IL6 concentrations correlated negatively with ultrasound measures of biparietal diameter and head circumference, whilst STAI state scores and maternal serum CRH concentrations correlated positively with abdominal circumference. Women with STAI state scores ≥40 in the 3rd trimester had babies with greater ultrasound measures of abdominal circumference and smaller ultrasound measures of head circumference compared to those with STAI state scores <40. Notably, maternal STAI state score during the 3rd trimester was the best positive predictor of ultrasound measures of abdominal circumference, and the best negative predictor of fetal head circumference and biparietal diameter. During pregnancy, a relative placental CRH-derived maternal hypercortisolemia develops from the 2nd trimester until birth. Thus, it appears that increased maternal acute stress (reflected by STAI state score) during the 3rd trimester of normal pregnancies, associates with increased measures of fetal abdominal circumference and decreased measures of fetal head circumference, indicating that maternal stress evaluated by subjective and objective markers during normal pregnancy affects fetal development. Maternal stress results in increased levels of serum cortisol, that in turn affects the fetus by overriding placental 11β-hydroxysteroid-dehydrogenase type 2 (Konstantakou et al., 2017; Yehuda et al., 2005; Rakers et al., 2015). This could have

detrimental effects on fetal cell proliferation, differentiation and synapse formation, mainly in amygdala, hippocampus and limbic cortical areas with short- and long- term postnatal consequences (Monteleone et al., 2014; Barros et al., 2006).

In one recent reported study on pregnant women, increased levels of maternal serum IL-6 and diurnal salivary alpha amylase (both markers of stress), were both associated negatively with head circumference at birth and positively with birthweight (Nazzari et al., 2019). In a further study on 2.6 million pregnancies in a Swedish population, the risk for low birth weight and small for gestational age fetuses was greater following acute stress exposure during the 2nd trimester of pregnancy (Class et al., 2011). A meta-analysis of 35 studies between 1991 and 2009 involving 31,323 women revealed a small, non-negligible, positive association between maternal psychosocial stress during pregnancy and risk for low birthweight (Littleton et al., 2010). It seems likely that the timing of maternal stress exposure during pregnancy could be an important factor that influences effects on fetal development. Taken together, our data suggest that greater acute maternal stress scores in the 3rd trimester of normal pregnancy affect fetal growth symmetry, by contributing directly and/or indirectly to decreased head circumference and increased abdominal circumference and birth waist circumference.

In our non-obese non-diabetic cohort of pregnant women, maternal chronic stress associated with increased fetal serum cortisol and insulin secretion (hyperinsulinemia). Maternal acute stress in the 3rd trimester associated with changes in fetal growth pattern including increased abdominal growth and decreased head growth. By influencing fetal growth and metabolism, maternal acute and chronic stress during pregnancy might predispose the fetus to IUGR, and subsequent development of enhanced HPA axis activity and insulin resistance. These pathophysiological observations might explain why babies with IUGR manifest increased susceptibility to development of type 2 diabetes later in life (Valsamakis et al., 2006). Furthermore, hormonal changes observed in umbilical cord blood might lead to epigenetic alterations in the offspring, thereby influencing fetal endocrine programming and brain development potentially across several generations (Stokes et al., 2012).

4.1. Conclusion

In conclusion, our study shows clear association between maternal acute- and long-term stress with patterns of fetal growth and metabolism. Chronic maternal stress seems to increase fetal cortisol, glucose, insulin, and c-peptide secretion, reflecting enhanced fetal insulin resistance. Further studies are required to elucidate the underlying pathophysiological mechanisms and interactions of maternal stress experienced during pregnancy, and the potential for epigenetic effects on future generations, through trans-generational predisposition to insulin resistance and HPA derangement.

Funding

Prof George Mastorakos received funding from Athens University. The funding source played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper

Author contribution paragraph: "G.V. designed the study, researched, analyzed and interpreted the data and wrote the manuscript; D.P., C.N., and M.M. researched data; I.P. and A.M. performed blood chemistry and revised the manuscript; S.K., T.M.B. and S.K. interpreted data and revised the manuscript; G.M. designed the study, interpreted data and reviewed the manuscript

References

Barros, V.G., Duhalde-Vega, M., Caltana, L., Brusco, A., Antonelli, M.C., 2006. Astrocyte-neuron vulnerability to prenatal stress in the adult rat brain. J Neurosci Res 83, 787-800.

Bischoff, S., Rupprecht, S., Kroegel, N., Schubert, H., Witte, O.W., Schwab, M., Rakers, F., 2018. Impact of chronic maternal stress during early gestation on maternal-fetal stress transfer and fetal stress sensitivity in sheep. Stress 21,1-10

Brunton, P.J., Sullivan, K.M., Kerrigan, D., Russell, J.A., Seckl, J.R., Drake, A.J.,2013 Sex specific effects of prenatal stress on glucose homeostasis and peripheral metabolism in rats. J Endocrinol 217, 161-73

Challis, J.R.G., Sloboda, D., Matthews, S.G., Holloway, A., Alfaidy, N., Patel, F.A, Whittle, W., Fraser, M., Moss, T.J.M, Newnham, J., 2001. The fetal placental hypothalamic–pituitary–adrenal (HPA) axis, parturition and postnatal health. Molecular and cellular Endocrinology 185, 135-144

Chrousos, G.P., Gold, P.W.,1992. The concepts of stress system disorders: overview of behavioral and physical homeostasis. JAMA, 267,1244–52).

Class, Q.A., Lichtenstein, P., Långström, N., D'Onofrio, B.M. 2011. Timing of prenatal maternal exposure to severe life events and adverse pregnancy outcomes: A population study of 2.6 million pregnancies Psychosom Med. 73, 234–241

DiPietro, J., Novak, M., Costigan, K.A., Atella, L.D., Reusing. S.P., 2006. Maternal Psychological Distress During Pregnancy in Relation to Child Development at Age Two. Child Development, Volume 77, Number 3, Pages 573 – 587

Dreiling, C.E., Carman, F.S., Brown, C.E., 1991. Maternal Endocrine and Fetal Metabolic Responses to Heat Stress J Dairy Sci 74, 312-327

Dreiling, M., Schiffner, R.,Bischoff, S., Rupprecht, S., Kroegel, N., Schubert, S., 2018. Impact of chronic maternal stress during early gestation on maternal–fetal stress transfer and fetal stress sensitivity in sheep Stress. 21:1-10.

Entringer, S., Wüst, S., Kumsta, R., Layes, I.M., Nelson, E.L., Hellhammer, D.H, Wadhwa, P.D., 2008. Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. Am J Obs&Gynae 188, 498.e1–498.e7

Entringer, S., Elissa S., Epelb P.S., Kumstac, R., Lind, J., Hellhammere, D.H., Blackburnd, E.H., Wüstf, S., Wadhwa, P.D., 2011. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood Proc Acad Natl USA 108, E513-8

Glover, V., Bergman, K., Sarkar, P., O'Connor, T.G., 2009. Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. Psychoneuroendocrinology. 34(3), 430-5

Flory, J.D., Bierer, L.M., Yehuda R., 2011 Maternal exposure to the holocaust and health complaints in offspring. Disease Markers. 30, 133–139

Jones, C.T., Gu, W., Parer, J.T., 1989. Production of corticotrophin releasing hormone by the sheep placenta in vivo. J. Dev. Physiol. 11, 97–101

Konstantakou, P., Mastorakos, G., Vrachnis, N., Tomlinson, J.W., Valsamakis, G.,2017 Dysregulation of 11beta-hydroxysteroid dehydrogenases: implications during pregnancy and beyond. J Matern Fetal Neonatal Med. 30, 284-293

Lesage, J., Del-Favero, F., Leonhardt, M., Louvart, H., Maccari, S., Vieau, D., Darnaudery, M., 2004. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. J Endocrinol. 81, 291–296

Li, J., Olsen, J., Vestergaard, M., Obel, K., Baker, J. L., Thorkild, I. A., Sørensen K., 2010 . Prenatal stress exposure related to maternal bereavement and risk of childhood overweight. PLoS One, 5,e11896

Littleton, H.L., Bye, K., Buck, K., Amacker, A,. 2010. Psychosocial stress during pregnancy and perinatal outcomes : a meta-analytic review J Psychosom Obstet Gynaecol 31, 219-28

Magiakou, M.A., Mastorakos, G,, Rabin, D,, Dubbert, B., Gold, P.W., Chrousos, G.P., 1996 Hypothalamic corticotropin-releasing hormone suppression during the postpartum period: Implications for the increase of psychiatric manifestations at this time J Clin Endocrinol Metab. 81, 1912-7

Mastorakos, G., Ilias, I.. 2000. Maternal hypothalamic-pituitary-adrenal axis in pregnancy and the postpartum period. Postpartum-related disorders. Ann N Y Acad Sci. 900, 95-106

Martin, J.A., Dodds, L., Tye, E., Arbuckle, A., Ettinger, S., Shapiro, G.D., Fisher, M., Taback, S., Bouchard, M.F., Monnier, P., Dallaire, R., Fraser. W.D., 2015 Maternal blood metal levels and fetal markers of metabolic function. Environ Res, 136, 27-34

Monteleone, M.C., Adrover, E., Pallares, M.E., Antonelli, M.C., Frasch, A.C., Brocco, M.A., 2014. Prenatal stress changes the glycoprotein GPM6A gene expression and induces epigenetic changes in rat offspring brain. Epigenetic 9: 152-156

Nazzari, S., Fearon, P., Rice, F., Dottori, N., Ciceri, F., Molteni, M., Frigerio, A., 2019. Beyond the HPA-axis: Exploring maternal prenatal influences on birth outcomes and stress reactivity. Psychoneuroendocrinol. ,101, 253-262

Paternain, L., de la Garza, A.L., Batlle, M.A., Milagro, F.I., Martínez, J.A., Campión, J., 2013. Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner. Stress. 16, 220-32.

Qian, S., Huifang, Z., Yanyan, Z., Huiping, Z., Ding, D., Junan, Z., Zhongliang, Z., Hui, Li., 2015. Maternal Stress in Gestation: Birth Outcomes and Stress-Related Hormone Response of the Neonates. Pediatrics and Neonatology 56, 376-381

Radaelli, T., Varastehpour, A., Catalano, P., Hauguel-de Mouzon, S., 2003. Gestational Diabetes Induces Placental Genes for Chronic Stress and Inflammatory Pathways. Diabetes 52, 2951-2958

Rakers, F., Bischoff, S.,, Schiffner, R.,, Haase., M, Rupprecht, S., Kiehntopf, M.,, Kühn-Velten., W.N., Schubert, H.,, Witte, O.W., Nijland, M.J., Nathanielsz, P.W., Schwab, M.,2015. Role of catecholamines in maternal-fetal stress transfer in sheep. Am J Obstet Gynecol. 213(5), 684.e1-9.

Rakers, F., Rupprecht, S., Dreiling, M., Bergmeier, C., Witte, O.W., Schwab, M. , 2017. Transfer of maternal psychosocial stress to the fetus. Neurosci Biobehav Rev. pii: S0149-7634(16)30719-9.

Romero-Gonzalez, B., Caparos-Gonzalez, R.A., Gonzalez-Perez, R., Delgado-Puertas, P., Peralta-Ramirez, M.I., 2018. Newborn infants hair cortisol levels reflect chronic maternal

stress during pregnancy. PLOS , 6,13, e0200279. doi: 10.1371

Rondó, P.H.C, Ferreira, R.F., Nogueira, F., Ribeiro, M.C.N., Lobert, H., Artes, R., 2003. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. European Journal of Clinical Nutrition 57, 266–272

Spielberger, C.D., Sydeman, S.J. 1994. State-Trait Anxiety Inventory and State-Trait Anger Expression Inventory. In M.E. Maruish (Ed.), The use of psychological testing for treatment planning and outcome assessment. (pp. 292-321). Hillsdale, NJ: Lawrence Erlbaum Associates

SPSS. SPSS for Windows 10.0.5 ed. Chicago Inc 1999

Stokes, T.A., Holston, A., Olsen, C., Choi, Y., Curtis, J., Higginson, J., Enright, L., Adimora, C., Hunt, C.E,. 2012. Preterm Infants of Lower Gestational Age at Birth Have Greater Waist Circumference-Length Ratio and Ponderal Index at Term Age than Preterm Infants of Higher Gestational Ages. J Pediatrics 16, 735-41

Teixeira, J.M.A., Fisk, N.M., Glover, V., 1979. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study BMJ. 318, 153–157

Valsamakis, G., Kanaka-Gantenbein, Ch., Malamitsi-Puchner, A., Mastorakos, G., 2006 Causes of intrauterine growth restriction and the postnatal development of the metabolic syndrome. Ann NY Acad Sci, 1092, 138-147

Valsamakis, G., Papatheodorou, D.C., Chalarakis, N., Vrachnis, N., Sidiropoulou, E.J., Manolikaki, M., Mantzou, A,, Margeli, A., Papassotiriou, I,, Chrousos, G.P., Mastorakos, G.,

2017. In pregnancy increased maternal STAI trait stress score shows decreased insulin sensitivity and increased stress hormones. Psychoneuroendocrinology, 84:11-16

Van den Bergh, B.R.H., van den Heuvel, M.I., Lahti, M., Braeken, M., de Rooij, S.R., Entringe,r S., 2017 . Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. Neurosci Biobehav Rev. https://doi.org/10.1016/j.neubiorev.2017.07.003 PMID: 28757456

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring.Brain Behavior and Immunity 19: 296–30

World Health Organization, 2013. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: WHO/NMH/MND/13.2; http://www.who.int/diabetes/publications/Hyperglycaemia_In_ Pregnancy/en/, accessed 29 September 2016

Yehuda, R., Engel, S.M., Brand, S.R., Seckl, J., Marcus, S.M., Berkowitz, G.S., 2005 Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy**.** J Clin Endocrinol Metab. 90, 4115-8.

Legend to the Figure

Figure 1: Correlation between cord blood glucose and maternal CRH concentrations in the 3rd trimester (p=0.027, r=0.655).

Table 1: Maternal anthropometric, hormone, metabolic and psychometric variables during pregnancy. Variables are expressed as mean (±SD) or median (25th–75th interquartile range); the asterisk (*) denotes statistically significant difference from the 1st trimester (P<0.05); (≠) denotes statistically significant difference (P<0.05) from the 2nd trimester.

Number of pregnancies	192		
MATERNAL VALUES			
Pre-pregnancy BMI (kg/m²)	26.9±2.4		
PREGNANCY	**1st trimester**	**2nd trimester**	**3rd trimester**
Weight (kg)	66 (±12.8)	70 (±12.7)*	79 (±14) *#
Systolic Blood pressure (mmHg)		107 (±12)	114 (±12)#
CRH (ng/ml)		2.2 (±0.4)	2.7 (±0.25)#
Cortisol (μg/dL)		22.2 (±6.7)	28 (±7.3) #
IL6 (ng/L)		1.9 (1.1-4)	1.6 (1.2-1.9)
STAI state		33.9 (±6.8)	40.34 (±6.65)#
STAI trait		39.6 (±8.4)	34.9 (±8.7)
FETAL VALUES			
US abdomen circumference (mm)		191.9 (±19.6)	289 (±22.3)#
US head circumference (mm)		218.6 (±20.7)	308.5 (±14.9)#
US estimated weight (gr)		650 (503–815)	2,270 (1,800–2,413)#
US biparietal diameter (mm)		58.6(±5.1)	84.3 (±5.4) #
NEONATAL VALUES			
Birthweight (gr)	3,075 (2,715-3,444)		
Birth waist (cm)	29.2 (±3.3)		
Cord cortisol (μg/dL)	11.1 (±9.7)		
Cord insulin (pmol/L)	10.5 (±5.4)		
Cord glucose (mg/dL)	99.3 (±10.13)		
Cord c-peptide (nmol/L)	0.8 (0.3)		

Table 2: Comparison of fetal U/S measured and neonatal anthropometric parameters according to maternal STAI state scores in the 3rd trimester (high: ≥40; low: <40).

	State<40	**State ≥40**	**p-value**
State score	30.6 (±4.01)	42.8 (±3.04)	0.034
US head circumference (mm)	315.3 (±7.2)	298.2 (±6.9)	0.023
U/S abdominal circumference (mm)	277.8 (±9.9)	301 (±10.6)	0.027
Birthweight (gr)	3,106 (2,898-3,564)	2,950 (2,702-3,352)	NS

Table 3: Comparison of fetal cord blood glucose, c-peptide and cortisol according to maternal STAI trait score in the 3rd trimester (high: ≥40; low: <40).

	Trait<40	**Trait≥40**	**p-value**
Trait score	32 (±5.6)	45.2 (±5)	0.032
Cord blood cortisol (µg/dL)	10 (±7.8)	15.1 (±8.6)	0.035
Cord blood glucose (mg/dL)	94 (±5.3)	103 (±6.4)	0.029
Cord blood c-peptide (nmol/L)	0.65 (0.15)	0.95 (0.15)	0.021

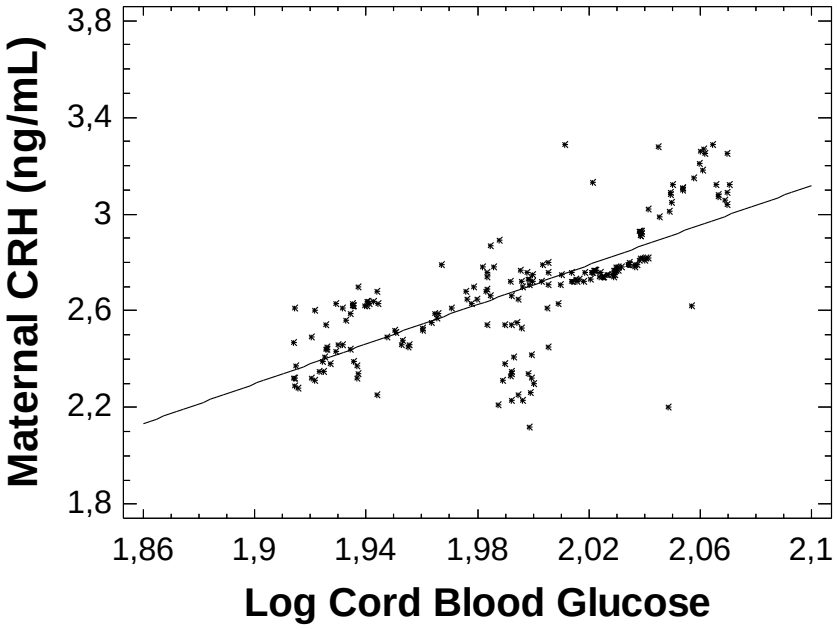
Table 4. Correlations between maternal IL6, CRH and cortisol concentrations and psychometric variables (STAI state and trait scores) with fetal ultrasonographic measurements in the 2nd trimester, birth waist, as well as with cord blood glucose, insulin, c-peptide and cortisol concentrations ($p<0.05$).

	Maternal IL6	**Maternal CRH**	**Maternal cortisol**	**STAI trait**	**STAI state**
Maternal CRH					0.455
Maternal cortisol		0.328			
Fetal US abdo			0.823		
Fetal US BPD		-0.357			
Birth waist	0.527	0.800	0.790		
Cord blood glucose				0.618	
Cord blood c-peptide				0.589	

Table 5. Correlations between maternal IL6, CRH and cortisol concentrations and psychometric variables (STAI state and trait scores) with fetal ultrasonographic measurements in the 3rd trimester, as well as with cord blood glucose, insulin, c-peptide and cortisol concentrations ($p<0.05$).

	maternal IL6	**Maternal CRH**	**Maternal cortisol**	**STAI state**	**STAI state ≥40**	**STAI trait**
Maternal CRH					r = 0.817	
Fetal US abdo circumference		0.396		0.548		
Fetal US head circumference	-0.865	-0.385		-0.362		
Fetal US BPD	-0.695	-0.355		-0.534		
Cord blood glucose		0.655	0.515			0.512
Cord blood insulin		0.679	0.586			0.517
Cord blood c-peptide		0.684	0.614			0.524
Cord blood cortisol		0.589				0.505

Figure 1


Maternal CRH (ng/mL)
3,8
3,4
3
2,6
2,2
1,8
1,86
1,9
1,94
1,98
2,02
2,06
2,1
Log Cord Blood Glucose