Second-Trimester Amniotic Fluid Corticotropin-Releasing Hormone and Urocortin in Relation to Maternal Stress and Fetal Growth in Human Pregnancy

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TITLE PAGE

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ABSTRACT

This study explored the association between the acute psychobiological stress response, chronic social overload and amniotic fluid corticotropin corticotropin-releasing hormone (CRH) and urocortin (UCN) in 34 healthy, second-trimester pregnant women undergoing amniocentesis. The study further examined the predictive value of second-trimester amniotic fluid CRH and UCN for fetal growth and neonatal birth outcome. The amniocentesis served as a naturalistic stressor, during which maternal state anxiety and salivary cortisol was measured repeatedly and an aliquot of amniotic fluid was collected. The pregnant women additionally completed a questionnaire on chronic social overload. Fetal growth parameters were obtained at amniocentesis using fetal ultrasound biometry and at birth from medical records. The statistical analyses revealed that the acute maternal psychobiological stress response was unassociated with the amniotic fluid peptides, but that maternal chronic overload and amniotic CRH were positively correlated. Moreover, amniotic CRH was negatively associated with fetal size at amniocentesis and positively with growth in size from amniocentesis to birth. Hardly any studies have previously explored whether acute maternal psychological stress influences fetoplacental CRH or UCN levels significantly. Our findings suggest that 1) chronic, but not acute maternal stress may affect fetoplacental CRH secretion and that 2) CRH is complexly involved in fetal growth processes as previously shown in animals.

KEYWORDS: Prenatal stress, psychobiological stress response, CRH, urocortin, salivary cortisol, fetal growth

INTRODUCTION

Prenatal stress has been linked to elevated levels of placental corticotropin-releasing hormone (CRH) in the maternal blood (Hobel, Dunkel-Schetter, Roesch, Castro, & Arora, 1990). Yet, contradictory findings exist as well (Petraglia et al., 2001). Both CRH and its close relative, urocortin (UCN), play prominent roles in human stress regulation and pregnancy.

CRH is released by the hypothalamus in response to stress. During pregnancy, the placenta likewise produces CRH, and placental mRNA expression in humans is detectable by seven weeks' gestation (Frim et al., 1988). The placenta secretes CRH predominantly into the maternal bloodstream causing maternal plasma levels to rise gradually from about 16 weeks' gestation with a more rapid increase setting in from approximately 24 and 30 weeks' gestation onwards (Campell et al., 1987; Golan, Jozak, & Conwell, 1994; Lockwood et al., 1996; McLean et al., 1995). Placental CRH reaches the maternal pituitary and stimulates adrenocorticotropic hormone (ACTH) and cortisol production. Cortisol, in turn, stimulates placental CRH secretion, which is contrary to the inhibitory effect it has on hypothalamic CRH (Robinson, Emanuel, Frim, & Majzoub, 1988). This feed-forward loop contributes to the well-known up-regulation of the maternal hypothalamic-pituitary-adrenal (HPA) axis in pregnancy.

The placenta releases small amounts of CRH into the fetal circulation, and levels in fetal plasma and amniotic fluid are measurable between 15-17 weeks' gestation (Gitau, Fisk, & Glover, 2004; Salminen-Lappalainen & Laatikainen, 1990). Fetal CRH levels are lower than and modestly correlated with maternal levels (Gitau et al., 2004; Smith, 2007). Studies to date have produced contradictory findings on whether fetal concentrations rise with advancing gestation. While some have not found any significant association between gestational age and fetal plasma CRH during the second half of gestation (Gitau et al., 2004; Lockwood et al., 1996) or between gestational age and amniotic CRH within the third trimester (Emanuel et al., 1994), others have reported a significant increase in amniotic CRH from the second trimester to birth (Laatikainen, Räisänen, & Salminen, 1988). Taken together, it is assumed that fetal CRH levels increase from early to late gestation (Smith, 2007), but probably at a slower pace than maternal levels.

Placental CRH stimulates the fetal HPA axis, fetal cortisol secretion, organ maturation and

growth (Grammatopoulos, 2008). However, increased concentrations are linked to growth restriction (Wadhwa et al., 2004). Moreover, placental CRH is associated with the timing of parturition. For example, maternal CRH concentrations during the early second trimester predict pre-, post-, and at term deliveries (McLean et al., 1995). However, it recently has been postulated that rather than causing growth restriction or preterm birth, elevated CRH concentrations reflect a placental response to adverse intrauterine conditions to enhance the flow of nutrients to the fetus and accelerate growth (Gangestad, Caldwell Hooper, & Eaton, 2012). If these heightened metabolic demands cannot be met anymore, birth onset is triggered.

UCN has biological homology to CRH and binds to CRH receptors and the CRH-binding protein (CRH-BP) (Florio, Vale, & Petraglia, 2004). The neuronal circuits of CRH and UCN are complexly interrelated and UCN appears to play a particular important role in stress adaptation (Kozicz, 2007). UCN is expressed in various reproductive tissues including the placenta (Florio et al., 2004), but has received little attention in prenatal stress research.

The key roles of CRH and UCN in stress and pregnancy and their high pharmacological resemblance underline the importance of studying both peptides more in depth. One underinvestigated question in this connection is whether the acute maternal stress response affects fetoplacental CRH and/or UCN levels (Thomson, 2013).

Amniotic CRH and UCN are of fetoplacental origin (Torricelli et al., 2009) and their levels can be determined during ongoing human pregnancy from amniotic fluid samples obtained at amniocentesis. Our workgroup has previously shown that this medical procedure elicits an anticipatory psychobiological stress response in pregnant women leading to significant higher levels of state anxiety, perceived stress, and salivary cortisol compared to a rest condition (Ghaemmaghami, Dainese, La Marca, Zimmermann, & Ehlert, 2014). In the current study, we therefore explored whether 1) the acute maternal psychobiological stress response and 2) chronic maternal stress are associated with amniotic CRH and/or UCN. We also examined whether 3) these peptides are related to fetal growth. We refrained from formulating a hypothesis for research question 1) due to its exploratory nature. However, based on the literature, we assumed that chronic stress would be associated with higher CRH and UCN levels. Conversely, we expected higher amniotic CRH and

UCN to be linked to lower fetal size and weight at amniocentesis and birth, shorter pregnancy duration, and increased catch-up growth.

MATERIALS AND METHODS

Overview

The current study is part of a larger prospective project on the psychobiological stress response in second-trimester pregnant women and their unborn children. The project details have been summarized previously (Ghaemmaghami et al., 2014; La Marca-Ghaemmaghami, Dainese, La Marca, Zimmermann, & Ehlert, 2015).

Participants

Healthy second-trimester pregnant women between the age of 18 and 45 years, with a singleton intrauterine pregnancy undergoing amniocentesis for karyotyping were eligible for the current study. The recruitment took place in cooperation with various hospitals in the German speaking part of Switzerland from April 2009 to December 2010. These included the Department of Gynecology and Obstetrics at the University Hospital of Zurich, the Cantonal Hospitals of Lucerne and Schaffhausen, the Hospitals of Buelach, Wetzikon, and Uster, and two private gynecological practices in Zurich and Winterthur.

Healthy pregnant women who had received an appointment for a second-trimester amniocentesis were informed about the ongoing study and asked whether they were interested in participating. Exclusion criteria were clarified during a phone screening interview prior to study inclusion and involved the following parameters: artificial insemination, known maternal and fetal medical complications, suspected or known fetal growth restriction, ultrasound confirmed fetal structural anomalies, maternal psychiatric disorder, smoking or alcohol consumption of more than one glass of wine or beer per week, medication use (e.g., glucocorticoids, psychotropic drugs, diuretics, antihypertensives, and vasodilators), and food and/or protein restrictions (e.g., vegetarian or vegan diet). Additionally, the pregnant women underwent a structured clinical interview during a laboratory visit using the computerized version of the Munich Composite Diagnostic Interview

(DIAX/M-CIDI) (Wittchen & Pfister, 1997) for the assessment of mental disorders according to DSM-IV in order to verify the absence of a psychiatric disorder. The interviews were conducted by trained clinical psychologists.

A total of 34 healthy pregnant women were included into the final sample. However, the amniotic fluid aliquot of one woman was contaminated with blood, and thus, excluded from statistical analyses for testing hypotheses involving amniotic fluid.

All women who were included into the current study had a normal amniocentesis test result and gave birth to a healthy child.

Procedure

All amniocenteses were scheduled for the morning hours due to the various hospital proceedings and logistics. The pregnant women were instructed to abstain from heavy physical exercise, chewing gum, caffeine and alcohol intake 24 hours prior to the amniocentesis appointment and to refrain from eating two hours, and from tooth brushing one hour beforehand. The women arrived at the hospital approximately 50 minutes prior to the amniocentesis procedure itself, gave written informed consent, and responded to study administrative questionnaires. Subsequently, the pregnant women repeatedly provided saliva samples and responded to psychological state questionnaires. They were examined by ultrasound prior to the amniocentesis procedure, and gestational age was determined by fetal ultrasound biometry. After the amniocentesis intervention, the pregnant women were still monitored for another 60 minutes in order to capture the entire stress recovery phase. The women received 200 Swiss Francs for their study participation, as well as a gift set containing skincare products for pregnancy, motherhood and infant care.

The study protocol was in accordance with the declaration of Helsinki and approval by the Ethics Committees of the Canton of Zurich, Schaffhausen, and Lucerne was acquired prior to the data collection phase.

Outcome measures

Psychological measures

Information on maternal state anxiety levels were obtained at -40 minutes and -10 minutes prior to, and then again at +20 minutes following the amniocentesis procedure by using the validated German version of the state subtest of the State-Trait Anxiety Inventory (STAI-s) (Laux, Glanzmann, Schaffner, & Spielberger, 1981; Spielberger, Gorsuch, & Lushene, 1970). Respondents are asked to describe their current feelings (e.g., '*I am tense*' or '*I feel nervous*') on a four-point Likert-type scale extending from (1) *not at all* to (4) *very much so*. Cronbach's alpha for the STAI-s lies at .91 (cf., Laux et al., 1981).

Maternal chronic social stress exposure was measured after the pregnant women had received notification of a normal amniocentesis test result by means of the social overload subscale of the Trier Inventory for Chronic Stress (TICS) (Schulz & Schlotz, 1999) – a widely used questionnaire to assess the experience of chronic stress. The respondents are asked to answer how often they have experienced various stressful situations in the past three months on a five-point Likert-type scale ranging from (0) *never* to (4) *very often*. The social overload subscale measures excessive demands a person takes for other people (e.g., '*I spend a lot of time dealing with other peoples' problems*' or '*I have work to do that involves carrying a lot of responsibility for other people.*'). The sum-scores can range from 0 (never having experienced social overload in the past three months) to 24 (having experienced social overload very often in the past three months). In a representative German sample, female participants reached a mean value of 7.8 (standard deviation = 5.1) on this subscale (Petrowski, Paul, Albani, & Brähler, 2012). Cronbach's alpha for this subscale ranges from .84 to .86 (Petrowski et al., 2012; Schulz, Schlotz, & Becker, 2004).

Biochemical measures

The saliva samples were collected at -1 minute prior to and again at +10, +20, +30, +45, and +60 minutes after the amniocentesis procedure by means of Salivettes (Sarstedt, Sevelen, Switzerland). The saliva samples were immediately stored at -20 °C until biochemical analysis.

Salivary cortisol was analyzed with a highly sensitive liquid chromatography–tandem mass

spectrometry (LC–MS/MS) method to overcome the problem of cross-reactivity with other similar substrates such as salivary cortisone (Perogamvros et al., 2009). There were three missing values due to insufficient saliva in the swabs of three different participants at the following time points: -1 minute, +10 minutes, and +20 minutes. These three missings were substituted with estimated values following Jönsson et al. (2010) by calculating the mean quotient (slope) between the missing and the subsequent values of all participants and multiplying this mean quotient with the respective pregnant woman's salivary cortisol value prior to the missing one (cf., Ghaemmaghami et al., 2014).

An aliquot of 2 ml of amniotic fluid was obtained for study purposes and immediately frozen at -80 °C until the biochemical analyses took place. Amniotic fluid CRH levels were analyzed with a CRH-radioimmunoassay (RIA) (cf., Stalla et al., 1989). The lower limit of detection (LOD) was 40pg/ml. The intra-assay coefficient of variance was 6.1% and the inter-assay coefficient of variance 7.9%. Amniotic fluid UCN was analyzed using the RK-019-14 Urocortin (Human) - RIA-Kit (range: 10-1280 pg/ml) developed by Phoenix Pharmaceuticals, Inc. Karlsruhe, Germany, with a rabbit anti-peptide serum. The lower LOD for amniotic fluid UCN was 20pg/ml.

Measures of fetal growth and neonatal birth outcome

Fetal size and weight were assessed at the amniocentesis procedure by means of fetal ultrasound biometry. Data regarding neonatal size and weight at birth, as well as length of gestation were obtained from medical records. We assessed fetal growth from amniocentesis to birth by subtracting fetal size and weight at the amniocentesis appointment from neonatal size and weight at birth.

Statistical Analyses

The statistical analyses were performed using SPSS (Chicago, IL, version 21) for windows.

For all analyses, values of amniotic fluid CRH and UCN below the LOD were set to LOD/√2 following recommendations for not highly skewed data by Hornung and Reed (1990). This was the case for 11 CRH and for none of the UCN values. Subsequent analyses showed no significant differences with regard to gestational age at amniocentesis between the women with amniotic CRH

values below the LOD (*Mdn* = 15.86) and those with values above the LOD (*Mdn* = 15.71, *U* = 107.00, *z* = -0.90, *p* = .376).

The area under the curve with respect to increase (AUCi) was computed for maternal state anxiety (STAI-s) and salivary cortisol responses using a trapezoid formula as previously described by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). However, in order to take the morning decline in salivary cortisol into account, a slightly modified trapezoid formula was calculated which included subtracting the skew AUC with respect to ground (AUCg) for the connection between the first and the last salivary measures (cf., Ghaemmaghami et al., 2014).

Potential associations between maternal psychological and biological parameters, amniotic fluid CRH and UCN measures, and fetal growth variables were explored using Kendall's *tau* for non-parametric data. Kendall's *tau* is recommended for the analyses of small data sets, as is the case in the present study, since it enables more accurate generalizations than Spearman's *rho* (Field, 2013) and is more robust to extreme observations (Wilcox, 2016). In order to compare effect sizes across studies, Kendall's *tau* was additionally converted to Pearson's *r* as recommended by Walker (2003).

Potential covariates were assessed prior to the main analyses based on bivariate correlations. Maternal sociodemographic and pregnancy-related variables (i.e., maternal age, education, body mass index (BMI), gestational age at amniocentesis, and parity) were unassociated with CRH and UCN ($p > .10$ for all variables, see Figure 1 for the non-significant association between gestational age at amniocentesis and CRH (Figure 1A) and UCN (Figure 1B), respectively).

- insert Figure 1 about here -

Covariates regarding fetal variables and birth outcome measures included a) gestational age at amniocentesis (correlated with fetal weight at amniocentesis ($\tau = .46$, $p < .001$, $r = .66$) and fetal size at amniocentesis ($\tau = .38$, $p = .004$, $r = .56$)), b) maternal BMI (correlated with length of gestation at birth ($\tau = .31$, $p = .020$, $r = .46$)), and c) length of gestation at birth adjusted for maternal BMI (correlated with neonatal weight ($\tau = .34$, $p = .008$, $r = .51$) and size at birth ($\tau = .31$, $p = .022$, $r =$

.46), and d) the period between amniocentesis and birth in weeks (correlated with fetal growth in weight from amniocentesis to birth (τ = .39, p = .003, r = .58), and fetal growth in size from amniocentesis to birth (τ = .31, p = .020, r = .47)). Therefore, a) gestational age-adjusted standardized residuals of fetal weight and size at amniocentesis, b) maternal BMI-adjusted standardized residuals of gestation length at birth c) gestational age at birth- and BMI-adjusted standardized residuals of neonatal weight and size, and d) number of weeks from amniocentesis to birth-adjusted standardizes residuals of fetal growth in weight and size from second-trimester amniocentesis to birth were calculated (cf., Pesonen et al., 2006). This procedure enables to overcome the effect of common variance between the individual maternal and fetal parameters.

The level of significance was set at $p < .05$ for all analyses. One-sided tests were used for directional hypotheses.

RESULTS

Sample characteristics

The descriptive characteristics of the study population, including maternal sociodemographic, health- and pregnancy-related variables, amniotic fluid parameters, and measures of fetal growth and birth outcome, are presented in Table 1.

- insert Table 1 about here -

The association between amniotic fluid CRH and UCN

Amniotic fluid CRH and UCN concentrations were positively correlated (τ = .23, p = .040, r = .36, one-sided).

The association between the acute maternal psychobiological stress response and amniotic fluid CRH and UCN

Maternal state anxiety (AUCi) was unassociated with amniotic fluid CRH (τ = –.05, p = .699, two-sided) and UCN concentrations (τ = .02, p = .872, two-sided).

Likewise, there was no significant association between the acute maternal cortisol response

(AUCi) and amniotic fluid CRH (τ = .09, p = .524, two-sided) or UCN levels (τ = –.04, p = .750, two-sided).

The association between maternal chronic social overload and amniotic fluid CRH and UCN

Pregnant women reported having experienced chronic social overload during the last three months at a median of 9.00 and a mode of 10.00 (range = 2.00-20.00). Chronic social overload correlated significantly and positively with amniotic fluid CRH (τ = .34, p = .012, r = .51, one-sided; see Figure 1). This result remained significant when the values under the LOD were excluded from the analysis (τ = .31, p = .049, r = .47, one-sided). Chronic social overload was unassociated with amniotic fluid UCN (τ = .21, p = .076, one-sided).

- insert Figure 2 about here -

The association between amniotic fluid CRH, UCN, and markers of fetal growth and neonatal birth outcome

Amniotic fluid CRH was negatively correlated with fetal weight (τ = –.23, p = .043, r = –.36, one-sided) and size (τ = –.23, p = .047, r = –.35, one-sided) at amniocentesis indicating that in women with higher amniotic fluid CRH concentrations, fetal weight and size tended to be lower. Amniotic fluid UCN levels were unassociated with fetal size (τ = –.09, p = .249, one-sided) or weight at amniocentesis (τ = –.07, p = .309, one-sided).

With regard to neonatal birth outcome, no significant associations were found between amniotic fluid CRH levels and gestational age at birth (τ = .04, p = .396, one-sided), neonatal birth weight (τ = .07, p = .305, one-sided), or birth size (τ = .06, p = .334, one-sided). However, higher amniotic fluid CRH concentrations were positively correlated with increased fetal growth in size from amniocentesis to birth (τ = .28, p = .024, r = .42, one-sided; see Figure 3). Again, this association was maintained when the statistical analyses were rerun without the samples with values

under the LOD (τ = .35, p = .024, r = .53, one-sided). No significant associations were found between amniotic fluid UCN levels and gestational age at birth (τ = .03, p = .422, one-sided), neonatal birth weight (τ = –.04, p = .391, one-sided), or birth size (τ = .01, p = .469, one-sided). Likewise, UCN was unassociated with fetal growth in weight (τ = -.06, p = .318, one-sided) and size (τ = .10, p = .239, one-sided) from amniocentesis to birth.

- insert Figure 3 about here -

DISCUSSION

In our sample of second-trimester pregnant women, the acute psychobiological stress response was neither related to amniotic CRH nor UCN. However, our analyses did reveal a positive correlation between chronic maternal social overload and amniotic CRH. Additionally, we examined whether both amniotic fluid peptides were linked to fetal growth and neonatal birth outcome. The results of our analyses showed a negative association between CRH and fetal weight and size at amniocentesis. Conversely, a positive association between CRH and fetal catch-up growth in size from amniocentesis to birth was found. Contrary to our hypothesis, no significant association between either amniotic CRH or UCN and neonatal birth outcome (i.e., gestational age, weight, and size at birth) was apparent.

Whether acute maternal stress affects fetoplacental CRH or UCN levels has hardly been investigated so far (Thomson, 2013), and our study aimed to contribute to the literature in this field. With regard to psychological stress reactivity, our results are in line with previous findings from similar studies. For instance, neither did Glover, Bergman, Sakar, and O'Connor (2009) nor did we (Ghaemmaghami et al., 2014) detect a significant association between maternal state anxiety and amniotic fluid cortisol levels. As to the physiological stress response, our findings suggest that a transient activation of the maternal HPA axis may not necessarily lead to significant increases in fetoplacental CRH or UCN concentrations. This result is in accordance with Gitau et al. (2004) who investigated the acute maternal and fetal stress response to intrauterine blood transfusion at the fetal intrahepatic vein, a medical procedure that causes pain to the fetus, since it involves needling the fetal

abdomen. As expected, the authors detected increases in fetal ACTH and cortisol, but surprisingly no changes in either fetal or maternal CRH levels. Protective biological mechanisms in the human placenta, such as CRH-BP or the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) may explain these findings. Both prevent CRH actions – either directly as in the case of CRH-BP or indirectly as in the case of 11β-HSD2. Biologically active maternal cortisol can pass the placenta where it has an excitatory effect on CRH secretion (Robinson et al., 1988). However, in the placental syncytiotrophoblast – the site of maternal-fetal exchange – 11β-HSD2 inactivates a large percentage of maternal cortisol into its inert metabolite, cortisone (Benediktsson, Calder, Edwards, & Seckl, 1997). Correspondingly, we previously found acute increases in maternal salivary cortisol to be positively correlated with amniotic cortisone (Ghaemmaghami et al., 2014). The placental 11β-HSD2 enzyme may, thus, indirectly also inhibit inadequate placental CRH stimulation when high maternal cortisol levels enter the placenta after acute stress. Rodent studies show that acute maternal stress causes an immediate upregulation of placental 11β-HSD2 activity by 160% (Welberg, Thrivikraman, & Plotsky, 2005). Prior chronic stress exposure, however, results in an impairment of this protective mechanism. Although it has long been uncertain whether a comparable process exits in humans, studies have recently begun to report similar findings in pregnant women (La Marca-Ghaemmaghami et al., 2015; O'Donnell et al., 2012).

Most studies examining associations between maternal chronic stress and placental CRH, measured this peptide in maternal blood and reported inconsistent findings (Chen et al., 2010; Hobel et al., 1999; Latendresse & Ruiz, 2010; Mancuso, Dunkel Schetter, Rini, Roesch, & Hobel, 2004; Petraglia et al., 2001; Tse, Rich-Edwards, Koenen, & Wright, 2012). The few studies that assessed amniotic CRH (Emanuel et al., 1994; Florio et al., 2008; Laatikainen et al., 1988; Menon, Arora, Hobel, & Fortunato, 2008; Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989; Torricelli et al., 2009) or UCN (Iavazzo et al., 2009; Imperatore et al., 2006; Torricelli et al., 2009) did not include any psychological stress variables. The current study, therefore, expands our knowledge and corroborates the theoretical assumption and evidence from previous investigations (Chen et al., 2010; Hobel et al., 1999; Latendresse & Ruiz, 2010; Mancuso et al., 2004; Tse et al., 2012) that maternal chronic stress leads to abnormal increases in fetoplacental CRH secretion. Chronically elevated

maternal cortisol is assumed to be one causal factor in this pathway. For instance, increased maternal cortisol at 15 weeks' gestation predicts elevated maternal CRH at 31 weeks (Sandman et al., 2006). Nevertheless, other mechanisms, such as increased maternal norepinephrine and epinephrine concentrations, which have been found to stimulate placental CRH release in vitro (Petraglia, Sutton, & Vale, 1989), or stress-induced alterations of the maternal immune system, uteroplacental blood flow, or oxygen delivery may also contribute to excessive placental CRH secretion.

Our result of a negative association between CRH and fetal weight and size at amniocentesis is in accordance with Goland et al. (1993) and Wadhwa et al. (2004). Both found higher CRH levels in maternal or umbilical cord blood to be linked to fetal growth restriction. However, the blood samples were obtained at later stages (i.e., at 31 weeks' gestation or directly after preterm birth) than our amniotic fluid samples were. Increased CRH levels do not seem to cause fetal growth restriction, yet conversely, growth restriction appears to reflect hostile intrauterine conditions to which the placenta responds to with greater CRH secretion in order to speed up fetal growth and secure survival (Gangestad et al., 2012). Remarkable examples of such responses have been observed in animal species. For example, the tadpoles of the Western Spadefoot toad show elevated hypothalamic CRH concentrations when their ponds begin to evaporate (Denver, 1997). This triggers a cascade of events that accelerates metamorphosis and enables the tadpoles to escape death. However, if CRH activity is experimentally blocked during pond desiccation, metamorphosis is halted (Denver, 1997). Similarly, pregnant women, who are exposed to a prolonged period without food intake of more than 13 hours, exhibit higher plasma CRH levels compared to unexposed pregnant women (Hermann, Siega-Riz, Hobel, Aurora, & Dunkel-Schetter, 2001). It is interesting to note that in humans, placental CRH complexly regulates the expression of glucose transporter proteins and thus, glucose availability for fetal growth (Gao et al., 2012). Our finding of a positive correlation between second-trimester CRH and fetal catch-up growth is in line with these observations, contributes to the existing literature, and encourages further research along this path.

Few studies have assessed the predictive value of second-trimester amniotic CRH and UCN for pregnancy duration and preterm delivery. The lacking association between CRH and gestational age at birth in our sample is consistent with Torricelli et al. (2009) who did not discover any

differences in second-trimester amniotic CRH levels between women with preterm and those with term births. Contrary to our results, the authors did observe lower UCN in women with preterm birth. This latter finding was contradicted by Iavazzo et al. (2009) and then again confirmed by Karaer et al. (2013). Second-trimester amniotic CRH and UCN might not yet yield reliable results regarding the timing of birth. Similarly, the predictive value of maternal plasma CRH for preterm delivery seems to reach clinical reliability only between 26-31 weeks' gestation (Sandman et al., 2006).

The lacking associations between maternal psychological stress variables, fetal growth parameters, and UCN in the current study should not discourage further exploration of this peptide when researching the underlying biological pathways of prenatal stress. Although CRH and UCN are similar in structure, they play diverging roles and exert their actions via different pathways. For instance, placental CRH concentrations increase with advancing gestation while UCN levels seem to remain stable (Florio et al., 2004). Furthermore, CRH has inflammatory properties and UCN anti-inflammatory (Torricelli et al., 2009).

For obvious ethical reasons, we were unable to examine changes in amniotic CRH and UCN levels over time following acute maternal stress. Our results are based on a single sample. Therefore, we cannot exclude the possibility that a different or longer timeframe is required to detect associations between acute stress and fetoplacental CRH and UCN.

The relative high mean age of 37.2 years in the current sample is common for pregnant women undergoing amniocentesis. However, this circumstance reduces the generalizability of the findings to younger pregnant women.

Despite the prospective study design, our analyses are correlative and cannot not imply causality. Additional assessment of CRH and UCN in maternal blood would have also been preferable, since the relationship between the peptides in plasma and amniotic fluid is poorly understood. Moreover, larger samples are desirable when replicating our results.

We assessed maternal chronic stress relying on the social overload scale of the TICS, which measures the psychological stress experienced in the past three months. Future studies would benefit from additionally examining indicators of maternal chronic stress from a biological perspective, such as analyzing cortisol concentrations in maternal hair or nail samples.

Careful attention should be given when selecting the technique for biochemical analyses. Studies with healthy pregnant women show an unusually wide range in amniotic CRH and UCN levels. For example, Salminen-Lappalainen and Laatikainen (1990) used radioimmunoassay (RIA) and reported mean CRH levels of 44.25 ± 4.28 pg/ml in women between 15-17 weeks' gestation. Torricelli et al. (2009) investigated women at 16 weeks and found mean concentrations of 1640 ± 680 pg/ml with quantitative colorimetric immunoassay. We measured a median CRH level of 69.00 pg/ml using RIA. The RIA findings emphasize the significance of the analysis method, since applying the same method results in comparable levels.

Concerning UCN, Iavazzo et al. (2009) used an enzyme-linked immunosorbent assay (ELISA) and reported mean levels of 1600 ± 490 pg/ml between 15.9-23.7 weeks' gestation. Using a specific and sensitive immunoenzymatic assay, Torricelli et al. (2009) found UCN levels at 16 weeks to range at 900 ± 260 pg/ml. Our amniotic UCN levels, like CRH, were measured with a RIA and revealed a mean of 60.6 ± 19.5 pg/ml. None of the other studies measured UCN with RIA.

Another issue concerns the relatively high LOD of UCN and CRH. However, in many studies investigating the second-trimester (e.g., Salminen-Lappalainen & Laatikainen, 1990; Torricelli et al., 2009), it is unclear whether the values below these specific LODs were included into the statistical analyses or not, and, if so, which methodical approach was selected. Such information needs to be documented more clearly, because diverging approaches could add to the unwanted variety in the reported peptide levels. In this study, the positive association between chronic social overload and amniotic CRH remained significant even when the samples under the LOD were excluded from the analyses. These same was true for the association between CRH and fetal catch-up growth in size from amniocentesis to birth.

Despite these limitations, the present findings substantially advance our understanding of the biological pathways of prenatal stress and suggest that chronic, but not acute maternal stress affects fetoplacental CRH. The current results also confirm recent assumptions that CRH plays a complex and dynamic role in the mechanisms of fetal growth.

AUTHORS' NOTE

Pearl La Marca-Ghaemmaghami and Sara M. Dainese share first authorship of this article. They contributed equally to this work.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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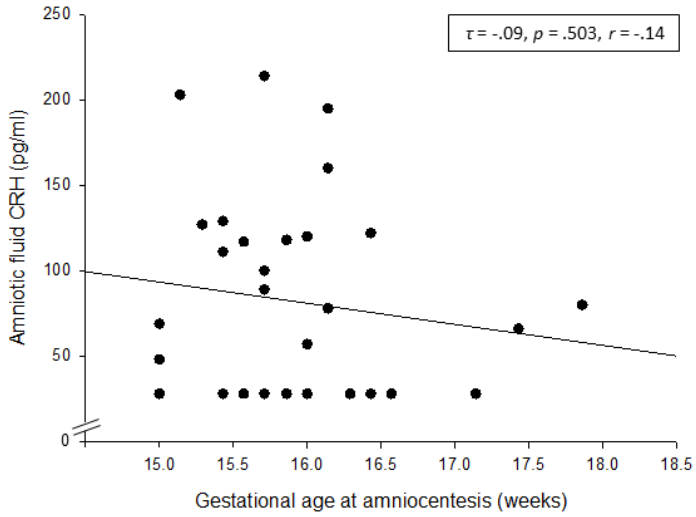
Table 1. Characteristics of the study population (N = 33).

At amniocentesis	
Maternal age (years) [mean ± SD (range)]	37.4 ± 4.1 (27-45)
Maternal education [% (N)]	
< 10 years	9.1 (3)
10-13 years	42.4 (14)
> 13 years	48.5 (16)
Maternal BMI (kg/m^2) [mean ± SD (range)]	22.8 ± 2.1 (19.6-27.3)
Gestational age (weeks) [mean ± SD (range)]	15.9 ± 0.7 (15.0-17.9)
Nulliparous [% (N)]	66.7 (22)
Amniotic fluid CRH (pg/ml) [median (range)]	69.0 (28.0-214.0)
Amniotic fluid UCN (pg/ml) [mean ± SD (range)]	60.6 ± 19.5 (25.0-100.0)
Fetal weight (gr) [median (range)]	138.0 (100.0-220.0)
Fetal size (cm) [median (range)]	17.2 (14.3-21.5)
Birth outcome measures	
Length of gestation (weeks) [mean ± SD (range)]	39.2 ± 1.4 (35.9-42.0)
Neonatal weight (gr) [mean ± SD (range)]	3293.2 ± 458.8 (1660.0-3960.0)
Fetal growth in weight from second-trimester amniocentesis to birth (gr) [mean ± SD (range)]	3149.9 ± 463.1 (1534.0-3804.0)
Neonatal size (cm) [median (range)]	49.0 (41.5-53.0)
Fetal growth in size from second-trimester amniocentesis to birth (cm) [mean ± SD (range)]	31.2 ± 2.8 (23.4-35.2)

Figure 1.

A



B

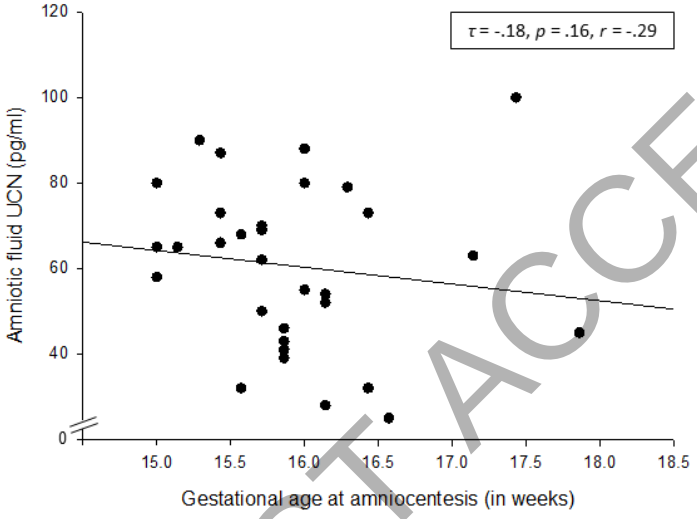


Figure 2.

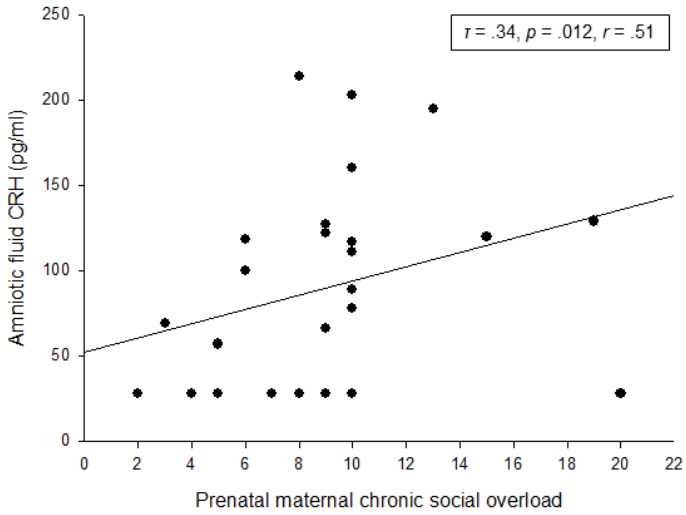


Figure 3.

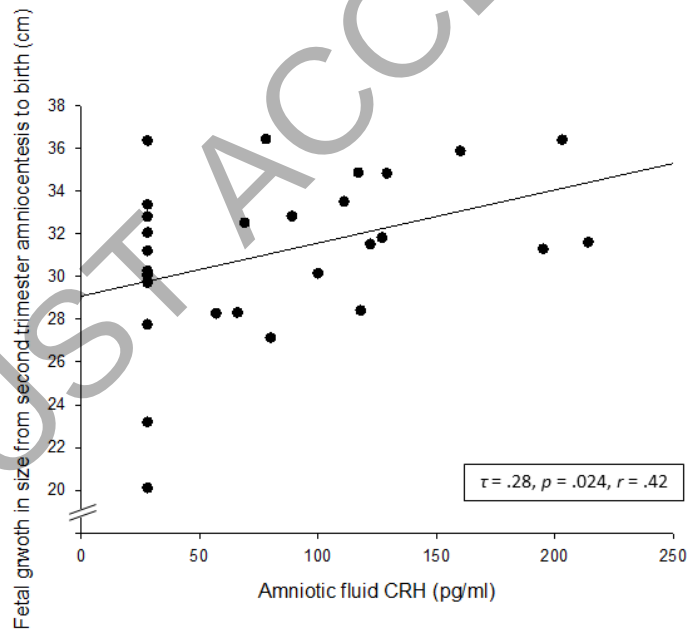


FIGURE CAPTIONS

Figure 1. **Bivariate correlation (Kendall's tau-b) between gestational age at amniocentesis (weeks) and amniotic fluid corticotropin releasing hormone (CRH) (A) and amniotic fluid urocortin (UCN) (B).** Results remain non-significant when samples with CRH values under the limit of detection (LOD) are excluded from the statistical analyses ($p > .10$ for both CRH and UCN).

Figure 2. **Bivariate correlation (Kendall's tau-b) between chronic social overload and amniotic fluid CRH in second-trimester pregnant women.** Results are maintained when samples with CRH values under the limit of detection (LOD) are excluded from the statistical analyses ($\tau = .31$, $p = .049$, $r = .47$, one-sided).

Figure 3. **Bivariate correlation (Kendall's tau-b) between amniotic fluid CRH and fetal growth in size from amniocentesis to term.** Fetal growth is adjusted for the period between amniocentesis and birth (in weeks) by using the standardized residuals of fetal growth in size. Results are maintained when samples with CRH values under the limit of detection (LOD) are excluded from the statistical analyses ($\tau = .35$, $p = .024$, $r = .53$, one-sided).

Note. Since the presentation of standardized residuals would reduce readability, we depict, for illustrative purposes only, fetal growth in size adjusted for the number of weeks from amniocentesis to birth as the sum between the mean of fetal growth across all participants and the individual standardized residuals.