



Year: 2015

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La Marca-Ghaemmaghami, Pearl ; Dainese, Sara M ; La Marca, Roberto ; Zimmermann, Roland ; Ehlert, Ulrike

Abstract: **OBJECTIVE:** The maternal autonomic nervous system (ANS) has received little attention in the investigation of biological mechanisms linking prenatal stress to fetal cortisol (F) excess. In vitro, norepinephrine and epinephrine inhibit placental 11 -hydroxysteroid dehydrogenase type 2 (11 -HSD2), which protects the fetus from F overexposure by inactivating it to cortisone (E). Here, we investigated the acute ANS stress response to an amniocentesis and its association with amniotic fluid F, E, and E/(E + F) as a marker of fetoplacental 11 -HSD2 activity. **METHODS:** An aliquot of amniotic fluid was obtained from 34 healthy, second-trimester pregnant women undergoing amniocentesis. Repeated assessment of mood states served to examine the psychological stress response to amniocentesis. Saliva samples were collected to measure stress-induced changes in salivary α -amylase concentrations in response to amniocentesis. Cardiac parameters were measured continuously. **RESULTS:** Undergoing amniocentesis induced significant psychological and autonomic alterations. Low-frequency (LF)/high-frequency (HF) baseline, suggested to reflect sympathovagal balance, was negatively correlated with amniotic E/(E + F) ($r = -0.53$, $p = .002$) and positively with F ($r = 0.62$, $p < .001$). In contrast, a stronger acute LF/HF response was positively associated with E/(E + F) ($r = 0.44$, $p = .012$) and negatively with F ($r = -0.40$, $p = .025$). **CONCLUSIONS:** These findings suggest that the maternal ANS is involved in the regulation of the fetoplacental barrier to stress. Allostatic processes may have been initiated to counterbalance acute stress effects. In contrast, higher LF/HF baseline values, possibly indicative of chronic stress exposure, may have inhibited 11 -HSD2 activity in the fetoplacental unit. These results parallel animal findings of up-regulated placental 11 -HSD2 in response to acute stress but impairment under chronic stress.

DOI: <https://doi.org/10.1097/PSY.000000000000130>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-102233>

Journal Article

Published Version

Originally published at:

La Marca-Ghaemmaghami, Pearl; Dainese, Sara M; La Marca, Roberto; Zimmermann, Roland; Ehlert, Ulrike (2015). The acute autonomic stress response and amniotic fluid glucocorticoids in second-trimester pregnant women. *Psychosomatic Medicine*, 77(1):41-49.

DOI: <https://doi.org/10.1097/PSY.000000000000130>

The Acute Autonomic Stress Response and Amniotic Fluid Glucocorticoids in Second-Trimester Pregnant Women

PEARL LA MARCA-GHAEMMAGHAMI, PhD, SARA M. DAINESE, PhD, ROBERTO LA MARCA, PhD, ROLAND ZIMMERMANN, MD, AND ULRIKE EHLERT, PhD

Objective: The maternal autonomic nervous system (ANS) has received little attention in the investigation of biological mechanisms linking prenatal stress to fetal cortisol (F) excess. In vitro, norepinephrine and epinephrine inhibit placental 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which protects the fetus from F overexposure by inactivating it to cortisone (E). Here, we investigated the acute ANS stress response to an amniocentesis and its association with amniotic fluid F, E, and E/(E + F) as a marker of fetoplacental 11 β -HSD2 activity. **Methods:** An aliquot of amniotic fluid was obtained from 34 healthy, second-trimester pregnant women undergoing amniocentesis. Repeated assessment of mood states served to examine the psychological stress response to amniocentesis. Saliva samples were collected to measure stress-induced changes in salivary α -amylase concentrations in response to amniocentesis. Cardiac parameters were measured continuously. **Results:** Undergoing amniocentesis induced significant psychological and autonomic alterations. Low-frequency (LF)/high-frequency (HF) baseline, suggested to reflect sympathovagal balance, was negatively correlated with amniotic E/(E + F) ($r = -0.53, p = .002$) and positively with F ($r = 0.62, p < .001$). In contrast, a stronger acute LF/HF response was positively associated with E/(E + F) ($r = 0.44, p = .012$) and negatively with F ($r = -0.40, p = .025$). **Conclusions:** These findings suggest that the maternal ANS is involved in the regulation of the fetoplacental barrier to stress. Allostatic processes may have been initiated to counterbalance acute stress effects. In contrast, higher LF/HF baseline values, possibly indicative of chronic stress exposure, may have inhibited 11 β -HSD2 activity in the fetoplacental unit. These results parallel animal findings of up-regulated placental 11 β -HSD2 in response to acute stress but impairment under chronic stress. **Key words:** prenatal stress, amniotic fluid, cortisone/cortisol ratio, 11 β -HSD2, autonomic nervous system, LF/HF HRV.

11 β -HSD1 = 11 β -hydroxysteroid dehydrogenase type 1; **11 β -HSD2** = 11 β -hydroxysteroid dehydrogenase type 2; **ANOVA** = analysis of variance; **ANS** = autonomic nervous system; **AUC_i** = area under the curve with respect to increase; **E** = cortisone; **EPI** = epinephrine; **F** = cortisol; **HF** = high-frequency component of heart rate variability; **HPA** = hypothalamic-pituitary-adrenal; **HR** = heart rate; **HRV** = heart rate variability; **LF** = low-frequency component of heart rate variability; **M** = mean; **Mdn** = median; **MMSQ** = Multidimensional Mood State Questionnaire; **NE** = norepinephrine; **sAA** = salivary α -amylase; **SD** = standard deviation; **SEM** = standard error of the mean; **UV** = ultraviolet.

INTRODUCTION

A body of evidence from animal and increasingly from human studies suggests that excessive psychological stress during pregnancy can adversely affect fetal development in utero and maternal and child well-being after birth (1,2). For example, increased prenatal maternal stress and anxiety have been associated with an altered activity of the hypothalamic-pituitary-adrenal (HPA) axis in neonates (3) and adolescents (4), with impaired cognitive development in infants (5), increased emotional and behavioral problems (6), and symptoms of attention-deficit hyperactivity disorder in children (7), and with shortened telomere length in young adults (8). These and other findings have led researchers to propose that similar to malnutrition during pregnancy,

maternal prenatal stress may exert programming effects on the developing fetus and thereby increase the individual's susceptibility to disease in later life (9).

To elucidate the underlying biological mechanisms by which prenatal maternal stress may reach the fetus, research has largely focused on the maternal and fetal HPA axes and the detrimental effects of fetal glucocorticoid excess. Glucocorticoid overexposure seems to impair the activity of the placental enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which converts hormonally active cortisol (F) into inactive cortisone (E) to shield the fetus from maternal F excess (10). Because of 11 β -HSD2, the F levels within the fetal system remain several fold lower than the F levels within the maternal system (e.g., ref. (11)). In rats, acute maternal stress induces an immediate up-regulation of placental 11 β -HSD2 activity, but after maternal chronic stress exposure, this up-regulation is impaired resulting in F excess to the fetus (12,13). A parallel process has been proposed to exist in humans because, from 5 weeks of gestation onward, 11 β -HSD2 is also present in the human placental syncytiotrophoblast, the site of maternal-fetal exchange (14). Support for this hypothesis comes from a recent study revealing a negative association between anxiety in pregnant women and 11 β -HSD2 activity and messenger RNA (mRNA) expression in placental samples obtained directly after birth (15).

Investigating placental 11 β -HSD2 activity in response to acute stress during ongoing human pregnancy poses ethical and medical challenges. This also applies to studies aiming at examining 11 β -HSD2 activity in vivo in other human tissues where the enzyme is highly expressed, such as the kidney. To do so, researchers have relied on the ratio between urinary free F and E as a surrogate marker for renal 11 β -HSD2 activity (16,17). This ratio has been found to correlate with the mRNA expression of 11 β -HSD2 in kidney tissues obtained from patients undergoing kidney biopsies (18). Hundertmark et al. (19) used the ratio between amniotic fluid F and E obtained at 21 to

From the Department of Psychology, Clinical Psychology and Psychotherapy (P.L.M.-G., S.M.D., R.L.M., U.E.), University of Zurich, Zurich, Switzerland; and the Department of Obstetrics (R.Z.), University Hospital Zurich, Zurich, Switzerland.

Address correspondence and reprint requests to Ulrike Ehlert, PhD, Department of Psychology, Clinical Psychology & Psychotherapy, University of Zurich, Binzmuehlestrasse 14/Box 26, CH-8050 Zurich, Switzerland. E-mail: u.ehlert@psychologie.uzh.ch

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.psychosomaticmedicine.org).

Received for publication March 21, 2014; revision received July 19, 2014.

DOI: 10.1097/PSY.0000000000000130

38 weeks of gestation as a marker of 11 β -HSD2 activity in the human fetal kidney because during this period of gestation, amniotic fluid is increasingly produced by fetal urination. Before this period—that is between 10 and 20 weeks of gestation—the composition of amniotic fluid largely resembles the composition fetal plasma, because the extracellular fluid can diffuse through the not-yet-keratinized fetal skin into the amniotic fluid (20). In the fetal membranes, 11 β -HSD2 has been detected from 8 weeks of gestation onward (21). However, the capacity of placental 11 β -HSD2 to metabolize F is assumed to exceed the capacity of fetal 11 β -HSD2 to metabolize F (22). On this basis, our workgroup previously examined F, E, and the E/(E + F) ratio in amniotic fluid samples obtained from second-trimester amniocenteses as a marker for 11 β -HSD2 activity in the fetoplacental unit (23). Our results showed that the maternal salivary F response was positively and significantly related to amniotic fluid E and at borderline significance to amniotic fluid E/(E + F). This finding indicates that the placental barrier to F functions in response to acute stress during ongoing human pregnancy, as it does in pregnant rats (23).

In comparison with the HPA axis, little attention has been paid to date to the potentially important role of the autonomic nervous system (ANS), which, like the HPA axis, is activated by stress. In vitro, placental 11 β -HSD2 is inhibited by norepinephrine (NE) and epinephrine (EPI) (24), highlighting the importance of also investigating this stress-sensitive system when exploring the underlying biological pathways of prenatal stress (9,15,25). To what extent the maternal ANS response may be associated with amniotic fluid F, E, and E/(E + F) is unknown. Plasma NE and EPI levels in nonpregnant participants have been associated with other ANS markers involved in the stress response, such as salivary α -amylase (sAA) and heart rate (HR) variability (HRV) (26,27), although findings remain inconsistent (28). The advantage of measuring the activity of these ANS markers in response to stress in pregnant women is that they can be investigated easily and noninvasively.

In the current study, we a) examined the stress response of different ANS markers (i.e., sAA, HR, and HRV) in pregnant women before, during, and after second-trimester amniocentesis compared with a control condition and b) explored whether these stress markers were associated with amniotic fluid F, E, and the E/(E + F) ratio as a marker of 11 β -HSD2 activity in the fetoplacental unit (10,22,29).

METHODS

Study Participants

Thirty-four healthy pregnant women with a singleton intrauterine pregnancy were examined while undergoing second-trimester amniocentesis for karyotyping. Participants were recruited at different Swiss hospitals after registering for amniocentesis. In Switzerland, testing for fetal chromosomal anomalies is routinely recommended to pregnant women 34 years and older. Also, because in Switzerland the mean age of women at maternity is 31.4 years (30), many pregnant women undergo prenatal genetic testing. In the present study, 20 pregnant women (58.8%) mentioned advanced age as a reason for amniocentesis (31). Other reasons given included a positive result in the first-trimester screening test (26.5%; $n = 9$), chromosomal anomalies in a previous pregnancy (2.9%; $n = 1$), and simply wanting to make sure that all is well with the fetus even without apparent evidence for an increased risk (11.8%; $n = 4$) (31).

None of the participants or their fetuses were affected by medical complications, and all participants had a normal amniocentesis result. Pregnancies via artificial insemination, current mental disorders, medication, smoking, or obesity were defined as exclusion criteria. Liquorice consumption was screened for as well, because its main ingredient, glycyrrhetic acid, is a well-known inhibitor of 11 β -HSD2 activity (32). None of the participants consumed liquorice during pregnancy. The study was carried out in accordance with the tenets of the Declaration of Helsinki, and ethical approvals were obtained by the cantonal ethics committees of Zurich, Schaffhausen, and Lucerne. All participants gave informed written consent.

Study Design and Procedure

The study at hand is part of a larger research project on the psychobiology of stress during human pregnancy (23,31). The study design is presented in Figure 1. Because of hospital logistics and proceedings, all amniocenteses were conducted during morning hours. Participants arrived approximately 50 minutes before the amniocentesis, filled in study administrative questionnaires, and were subsequently asked to put on an ambulatory electrocardiographic device before being seated in a semirecumbent chair. Cardiac activity was continuously measured, and repeated saliva samples and psychological measurements were obtained. Gestational age was determined by fetal ultrasound biometry before the amniocentesis. After the amniocentesis, participants were still monitored for 60 minutes to capture the stress recovery period. We invited the pregnant women to participate in a control condition after they had received the notification of a normal amniocentesis test results (mean [M; standard deviation {SD}] = 2.74 [1.00] weeks after the amniocentesis). All 34 pregnant women participated. The procedure of the control condition was identical to the amniocentesis condition, except that no amniocentesis took place. It was scheduled at the same time as the amniocentesis condition to control for circadian fluctuations.

Outcome Measures

Psychological Measures

Maternal mood was assessed before and after the amniocentesis at -40 , -10 , and $+20$ minutes (see Fig. 1) and at equivalent times during the control condition using the validated 24-item German version of the Multidimensional Mood State Questionnaire (MMSQ) (33). The MMSQ is composed of three subscales: “good-bad mood” (Cronbach $\alpha = .91-.94$), “calmness-nervousness”

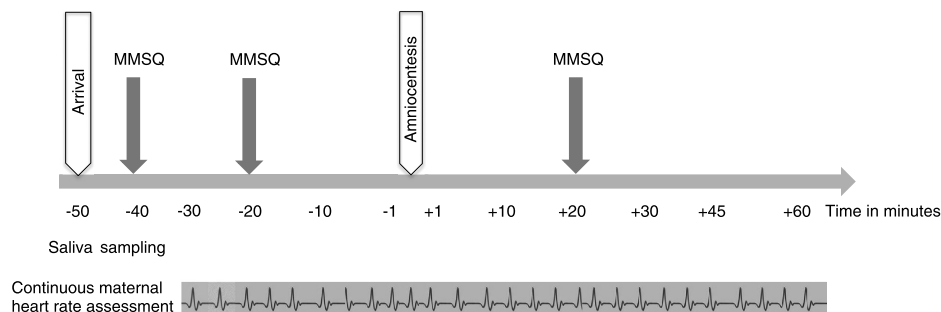


Figure 1. Study design and procedure. MMSQ = Multidimensional Mood State Questionnaire.

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(Cronbach $\alpha = .92-.96$), and “wakefulness-tiredness” (Cronbach $\alpha = .86-.91$). Participants are asked to rate to what extent they, for example, feel “content”, “restless, or “worn-out” on a 5-point Likert scale (1= definitely not, 5 = extremely). Higher scores indicate good mood, calmness, and wakefulness.

Biochemical Measures

Salivary α -Amylase

Saliva samples were collected with Salivettes (Sarstedt, Sevelen, Switzerland) at -20, -10, -1, +1, +10, +20, +30, +45, and +60 minutes. The saliva samples were stored at -20°C until biochemical analysis. After thawing, sAA activity was analyzed with the automatic analyser Cobas Mira and assay kits from Roche (27) with intra-assay and interassay coefficients of variation lower than 8%.

Because of insufficient saliva, a total of eight missing values from seven different participants were replaced, following Jönsson et al. (34), by calculating the slope between the missing and the subsequent value across all participants and multiplying it by the value of the individual participant preceding the missing one.

Marker of 11 β -HSD2 Activity

Amniotic fluid parameters: a surplus of 2 ml amniotic fluid was obtained and stored at -80°C. F and E were analyzed using reversed-phase high-performance liquid chromatography with UV detector. The intra-assay and inter-assay coefficients of variation are shown to be lower than 5% for both F and E in urine and plasma. Intra-assay coefficients of variation for amniotic fluid F and E have been found to be lower than 3% (35).

11 β -HSD2 activity in the fetoplacental unit was assessed in percentage using the subsequent formula: $E/(E + F) * 100$ (cf. Refs. (10,22,29)). In the following, this variable is reported as $E/(E + F)$ for brevity's sake.

Electrophysiological Measures

Cardiac activity was measured using the LifeShirt system 200 (Vivometrics, Ventura, CA) (36). Five-minute intervals of raw data were edited manually and corrected for ectopic beats and arrhythmias by applying linear interpolations. These selected intervals began at -32.5, -17.5, -5, +12.5, +32.5, and +47.5 minutes. After applying fast Fourier transformation, the corrected interbeat intervals were analyzed for HR and frequency domain measures of HRV, that is, high-frequency (HF; 0.15-0.4 Hz, mirroring parasympathetic modulation of HR and cardiac vagal activity) and low-frequency (LF; 0.04-0.15 Hz, mirroring both sympathetic and parasympathetic modulation). The ratio between the two (LF/HF) is thought to reflect sympathovagal balance, but this concept has also provoked controversy (37,38). Prior studies have observed a decreased HF and increased LF/HF ratio in response to psychosocial stress in both second-trimester pregnant and nonpregnant women alike, whereas no significant changes were found for LF (39), indicating a predominant vagal response to acute psychological stress.

Data Analyses

Data were tested for normal distribution, whereupon sAA, HF, LF, LF/HF, and amniotic fluid E underwent natural log transformation (ln) due to skewness. Then, to ensure the comparability of variables when conducting analyses to explore potential associations between maternal ANS and amniotic fluid parameters, previously untransformed variables (i.e., HR and amniotic fluid F) underwent natural log transformation as well. This procedure is in line with Field (40). Both maternal HR and amniotic fluid F remained normally distributed after transformation. The $E/(E + F)$ ratio was calculated with the untransformed E and F values, as it constitutes a specific formula in percentage and was normally distributed.

Two-way repeated-measures analyses of variance (ANOVAs) were computed to identify possible interaction effects between the amniocentesis and control condition. Wherever the assumption of sphericity was violated, results underwent Greenhouse-Geisser correction. Values for the area under the curve with respect to increase (AUCi) were computed using the trapezoid formula (41) while additionally controlling for participants' individual baseline value (last measurement point of the study protocol). Comparisons between the two conditions were analyzed with paired Student's *t* tests. For post hoc testing, ANOVAs were conducted for each condition separately and Bonferroni tests were computed to examine possible differences between the individual time points.

With regard to psychological measures, nonnormal distributions were found for the good-bad mood and calmness-nervousness subscales of the MMSQ. Therefore, nonparametric tests were used for these two scales. Possible differences between the conditions were examined with the Wilcoxon signed rank test. Effects of time were investigated using Friedman ANOVA.

Potential associations between maternal psychological, ANS, and amniotic fluid parameters were explored using bivariate and partial correlations. Control variables were determined before the correlation analyses by conducting bivariate correlations (Pearson and Kendall τ correlations) between maternal characteristics and untransformed maternal physiological markers. These control variables included gestational age (associated with baseline HR: $r = 0.48, p = .011$; baseline HF: $\tau = -0.41, p = .001$; baseline LF/HF: $\tau = 0.27, p = .022$; sAA reactivity: $\tau = 0.24, p = .055$; and amniotic fluid F: $r = 0.36, p = .044$) and maternal body mass index (associated with HR reactivity: $r = -0.34, p = .047$).

All analyses were two tailed. The level of significance was set at $p < .05$.

RESULTS

Sample Characteristics

Sociodemographic and pregnancy-related characteristics of the participants are presented in Table 1.

Effects of the Amniocentesis

Maternal Psychological Stress Response

Results for the good-bad mood subscale of the MMSQ indicated that *mood* was significantly worse at -40 and -10 minutes of the amniocentesis compared with the control condition (Wilcoxon signed rank test at -40 minutes: amniocentesis: median [Mdn] = 34.50; control condition: Mdn = 38.00; $z = -2.72, N\text{-Ties} = 29, p = .005, r = -0.33$; Wilcoxon signed rank test at -10

TABLE 1. Sociodemographic and Pregnancy-Related Characteristics ($n = 34$)

Maternal age, M (SD) [range], y	37.38 (4.02) [27.00–45.00]
Marital status, % (<i>n</i>)	
Single	32.4 (11)
Married	58.8 (20)
Divorced	8.8 (3)
Living with partner in same household, % (<i>n</i>)	82.4 (28)
Ethnicity, % (<i>n</i>)	
European	82.4 (28)
Asian	14.7 (5)
African Caribbean	2.9 (1)
Education, % (<i>n</i>)	
<10 y	8.8 (3)
10–13 y	41.2 (14)
>13 y	50 (17)
Working time, M (SD) [range], %	69.71 (40.04) [0–100]
Annual income (Swiss Francs), % (<i>n</i>)	
<40,000	32.4 (11)
40,000–60,000	8.8 (3)
>60,000	52.9 (18)
BMI at amniocentesis, M (SD) [range], kg/m ²	22.66 (2.14) [19.60–27.30]
Gestational age at amniocentesis, M (SD) [range]	15.96 (0.71) [15.00–17.86]
Nulliparous, % (<i>n</i>)	64.7 (22)

M = mean; SD = standard deviation; BMI = body mass index.

minutes: amniocentesis: Mdn = 34.00; control condition: Mdn = 38.00; $z = -4.40$, N -Ties = 29, $p < .001$, $r = -0.53$) but not at +20 minutes (Wilcoxon signed rank test at +20 minutes: amniocentesis: Mdn = 36.00; control condition: Mdn = 37.50; $p = .055$; Fig. 2A).

Mood varied across time during the amniocentesis at marginal significance (Friedman ANOVA: $\chi^2(2) = 5.41$, $p = .066$), but not during the control condition ($\chi^2(2) = 3.35$, $p = .19$).

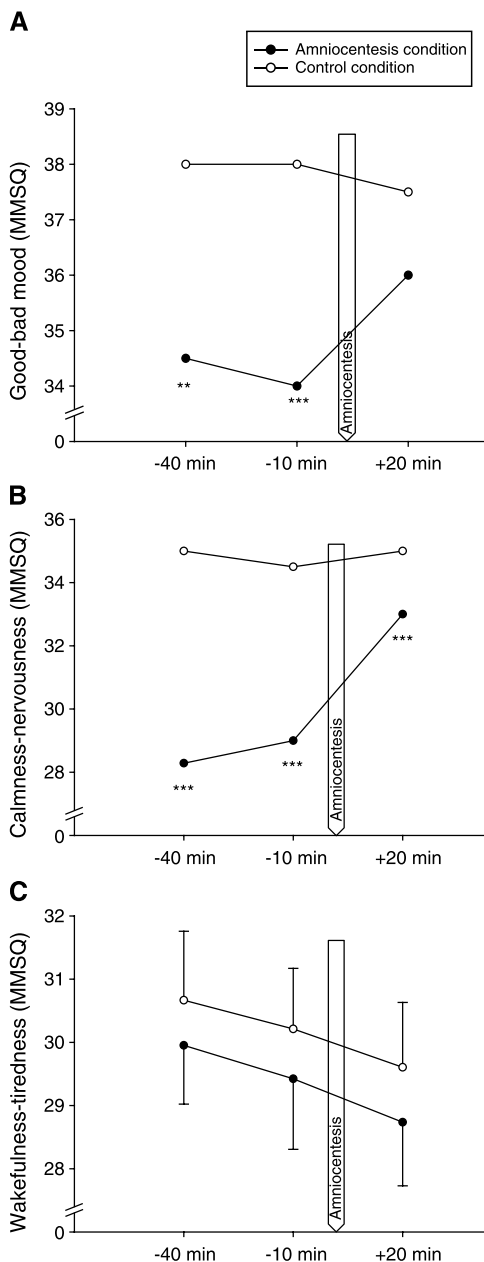


Figure 2. Psychological stress response of second-trimester pregnant women across time during the amniocentesis and control condition measured with the “good-bad mood” (median; A), “calmness-nervousness” (median; B), and “wakefulness-tiredness” (mean [standard error of the mean]; C) subscales of the MMSQ. Higher scores indicate increased good mood, calmness, wakefulness. ** $p < .01$, *** $p < .001$. MMSQ = Multidimensional Mood State Questionnaire.

Results for the calmness-nervousness subscale of the MMSQ showed significantly decreased calmness scores at all times points during the amniocentesis compared with the control condition (Wilcoxon signed rank test at -40 minutes: amniocentesis: Mdn = 38.29; control condition: Mdn = 35.00; $z = -4.31$, N -Ties = 32, $p < .001$, $r = -0.52$; Wilcoxon signed rank test at -10 minutes: amniocentesis: Mdn = 29.00; control condition: Mdn = 34.50; $z = -4.19$, N -Ties = 29, $p < .001$, $r = -0.50$; Wilcoxon signed rank test at +20 minutes: amniocentesis: Mdn = 33.00; control condition: Mdn = 35.00; $z = -3.50$, N -Ties = 29, $p < .001$, $r = -0.42$; Fig. 2B)

Calmness-nervousness varied significantly across time during the amniocentesis condition (Friedman ANOVA: $\chi^2(2) = 9.29$, $p = .009$), but not during the control condition ($\chi^2(2) = 3.41$, $p = .18$).

The two-way repeated-measures ANOVA for the wakefulness-tiredness scale revealed no significant differences between the conditions ($F(1,32) = 1.31$, $p = .26$). However, significant changes across time were apparent ($F(2,64) = 3.45$, $p = .038$, $\eta^2 = 0.10$). The interaction effect failed to reach significance (interaction condition by time: $F(2,64) = 0.01$, $p = .99$), suggesting that wakefulness decreased across time in both conditions (Fig. 2C).

Maternal Salivary α -Amylase Response

The amniocentesis led to a significant increase in sAA over time compared with the control condition (interaction condition by time: $F(5.05,156.63) = 6.80$, $p < .001$, $\eta^2 = 0.18$; see Fig. 3A). The AUCi of the amniocentesis condition ($M = 23.54$, standard error of the mean [SEM] = 7.46) was significantly larger compared with the control condition ($M = -6.55$, SEM = 6.01, $t(31) = 3.02$, $p = .005$, $r = 0.48$). Post hoc ANOVAs revealed a significant effect of time across the amniocentesis ($F(5.06,161.86) = 7.68$, $p < .001$, $\eta^2 = 0.19$) but also across the control condition ($F(4.33,138.59) = 3.15$, $p = .014$, $\eta^2 = 0.09$). The Bonferroni post hoc test revealed an sAA increase during the control condition from -1 to +45 minutes ($p = .005$).

Maternal Cardiac Response

HR increased significantly during the amniocentesis compared with the control condition (interaction condition by time ($F(3.46,110.81) = 13.37$, $p < .001$, $\eta^2 = 0.30$; see Fig. 3B). This finding was confirmed by differences in the AUCi values between the conditions (amniocentesis condition: $M = 474.00$, SEM = 51.39; control condition: $M = 285.69$, SEM = 78.52, $t(32) = 2.20$, $p = .035$, $r = 0.48$). Post hoc ANOVAs yielded a significant time effect for the amniocentesis ($F(3.41,119.25) = 37.56$, $p < .001$, $\eta^2 = 0.52$) but also for the control condition ($F(3.01,96.23) = 4.72$, $p = .004$, $\eta^2 = 0.13$) because of a significant decrease in HR during the control condition from -30 to +50 minutes ($p = .002$).

HF increased significantly during the amniocentesis compared with the control condition (interaction condition by time ($F(5,160) = 3.87$, $p = .002$, $\eta^2 = 0.11$; see Fig. 3C). This result was further substantiated by differences in the AUCi values (amniocentesis: $M = -10.20$, SEM = 6.45; control condition: $M = -34.11$, SEM = 6.25, $t(32) = 2.83$, $p = .014$, $r = 0.45$). Post

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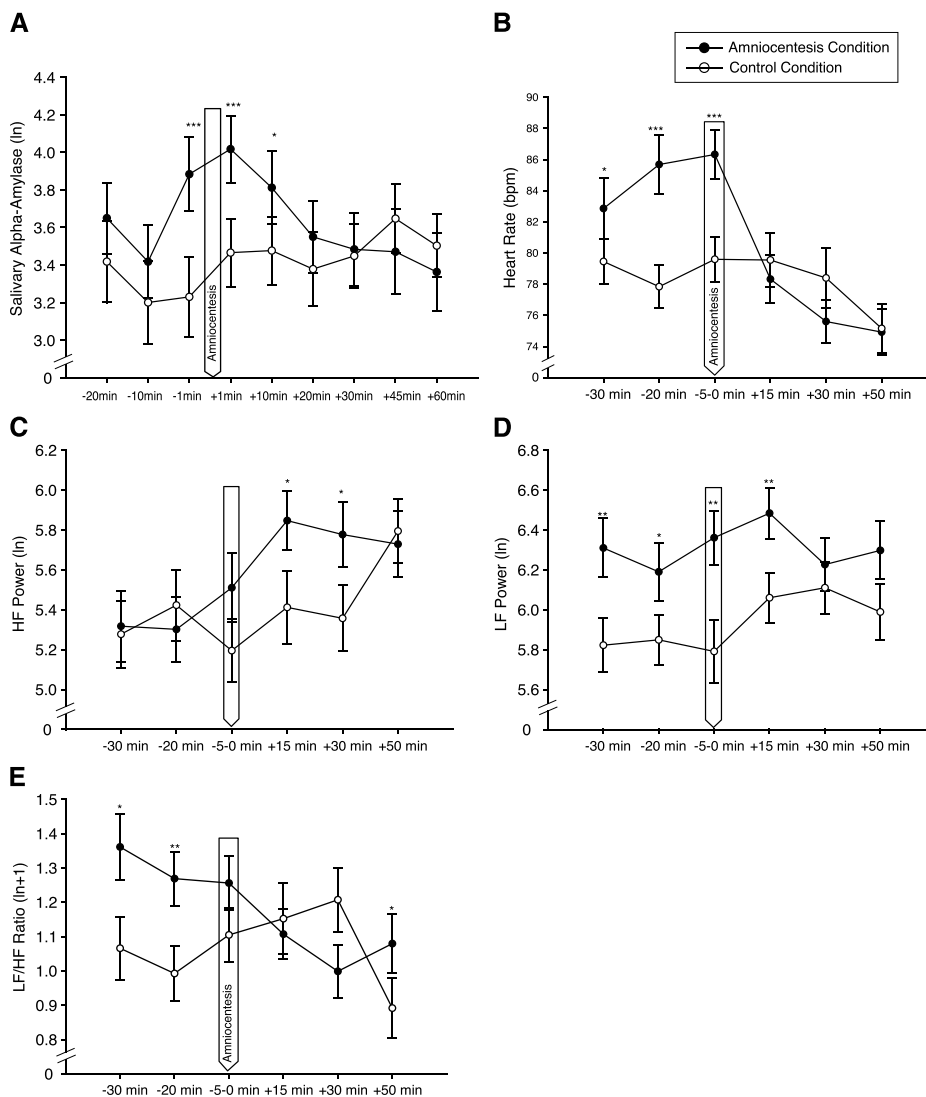


Figure 3. Stress response (mean and standard error of the mean) of salivary α -amylase (A), heart rate (B), high-frequency power (C), low-frequency power (D), and the LF/HF ratio (E) during the amniocentesis condition compared with the control condition. * $p < .05$, ** $p < .01$, *** $p < .001$. HF = high-frequency component of heart rate variability; LF = low-frequency component of heart rate variability.

hoc ANOVAs showed significant time effects for both conditions (amniocentesis: $F(3.51,119.31) = 9.10, p < .001, \eta^2 = 0.21$; control condition: $F(3.78,117.29) = 6.57, p < .001, \eta^2 = 0.18$). Bonferroni post hoc tests revealed a significant increase from -30 to $+50$ minutes during the control condition ($p = .001$), as well as from -5 to $+50$ minutes ($p < .001$).

LF findings revealed neither a significant interaction effect ($p = .15$) nor a significant difference between the AUCi values ($p = .59$; Fig. 3D). However, a significant effect of condition was apparent ($F(1,32) = 15.92, p < .001, \eta^2 = 0.33$), indicating that LF values were overall increased during the amniocentesis compared with the control condition.

LF/HF decreased significantly during the amniocentesis compared with the control condition ($F(5,160) = 5.18, p < .001, \eta^2 = 0.14$; see Fig. 3E). There was no significant difference between the two AUCi values ($p = .23$). Post hoc ANOVAs revealed a significant time effect in both conditions (amniocentesis: $F(3.97,138.91) = 6.01, p < .001, \eta^2 = 0.15$; control

condition: $F(5,160) = 3.19, p = .009, \eta^2 = 0.09$) because of a marginally significant decrease from $+30$ to $+50$ minutes ($p = .055$) in the control condition.

Association Between the Maternal Psychological, ANS, and Amniotic Fluid Variables

Wakefulness was positively associated with HR baseline levels ($r = 0.37, p = .032$). Aside from this result, psychological stress variables were unrelated to the maternal ANS and amniotic fluid variables (Supplemental Digital Content 1, <http://links.lww.com/CONT/A135>).

Association Between Maternal ANS and Amniotic Fluid Parameters

Association Between Maternal ANS Baseline Levels and Amniotic Fluid F, E, E/(E + F)

A negative association was found between the LF baseline and amniotic fluid E/(E + F) ($r = -0.36, p = .042$), and a

positive association was revealed between LF baseline and amniotic fluid F ($r = 0.40, p = .026$). Moreover, the LF/HF baseline was negatively correlated with amniotic fluid E/(E + F) ($r = -0.53, p = .002$; see Fig. 4A) and positively amniotic fluid F ($r = 0.62, p < .001$). No significant associations were found between sAA, HR, and HF baseline values and any of the amniotic fluid parameters (Supplemental Digital Content 2, <http://links.lww.com/CONT/A136>).

The ANS Stress Response and Amniotic Fluid F, E, and E/(E + F)

A trend toward significance was apparent for a positive relationship between the sAA AUCi and amniotic fluid E/(E + F) ($r = 0.36, p = .055$) and for a negative association between HF and amniotic fluid E/(E + F) ($r = -0.33, p = .062$). No significant associations were found between LF and amniotic fluid E or E/(E + F), but a negative relationship was apparent for amniotic fluid F ($r = -0.37, p = .039$). The LF/HF AUCi was positively related to amniotic fluid E/(E + F) ($r = 0.44, p = .012$; see Fig. 4B) and negatively to amniotic fluid F ($r = -0.40, p = .025$) (Supplemental Digital Content 3, <http://links.lww.com/CONT/A137>).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the association between the maternal ANS stress response and amniotic fluid parameters during human pregnancy. The amniocentesis provoked a significant anticipatory psychobiological stress response in second-trimester pregnant women with a decrease in mood and increases in nervousness, sAA, and HR. HF was decreased before the amniocentesis and started to rise again, whereas the opposite was true for LF/HF. No significant stress-induced LF changes were revealed, although LF levels were overall significantly higher during the amniocentesis compared with the control condition. The maternal psychological response to the amniocentesis was largely unassociated with the maternal ANS and amniotic fluid parameters, except that maternal wakefulness correlated positively with maternal HR baseline levels. Regarding the association between the maternal ANS response and amniotic fluid parameters, results showed that LF and LF/HF baseline values were negatively related to amniotic fluid

E/(E + F). The E/(E + F) ratio served as a marker of 11 β -HSD2 activity in the fetoplacental unit. Also, LF and LF/HF baseline levels were positively associated with amniotic fluid F. As for the acute stress response, LF/HF was positively related to amniotic fluid E/(E + F) and both LF and LF/HF were negatively associated with amniotic fluid F. Furthermore, correlations at borderline significance were found between the acute sAA response and amniotic fluid E/(E + F) (positive association) and between the acute HF response and amniotic fluid E/(E + F) (negative association).

Compared with the HPA axis, little attention has been paid so far to the role of the maternal ANS in the research of prenatal stress. Especially the investigation of sAA and HRV in response to acute psychological stress during pregnancy is limited (39,42,43). Salivary glands are innervated by both branches of the ANS, and stress-induced sAA changes are thought to reflect sympathetic activity (44), yet findings remain uncertain to whether parasympathetic modulation may be involved as well (28). Our results may, therefore, indicate increased sympathetic activity during the amniocentesis, but further research is needed. These findings parallel previous results from our workgroup on nonpregnant healthy participants (27,45) and are in line with our prior study on sAA reactivity during pregnancy revealing an increase in second- and third-trimester pregnant women confronted with an acute laboratory-based psychosocial stressor (42).

Cardiac autonomic activity seemed to exhibit an anticipatory stress response, as the increased HR and LF/HF levels before the amniocentesis suggest. Likewise, HF (i.e., vagal activity) was decreased 20 minutes before the amniocentesis and began to rise again. This poststress vagal rebound (46) reached significance 15 minutes after the amniocentesis before returning to levels comparable with the control condition. This suggests that sympathetic activity may have predominated already before the amniocentesis.

In line with previous results (39), we found no significant LF changes over time, although levels were overall significantly higher during the amniocentesis condition. Possibly, sympathetic tone was increased during the entire amniocentesis condition, yet the validity of this interpretation remains uncertain, because LF is influenced by both vagal and sympathetic activity (37).

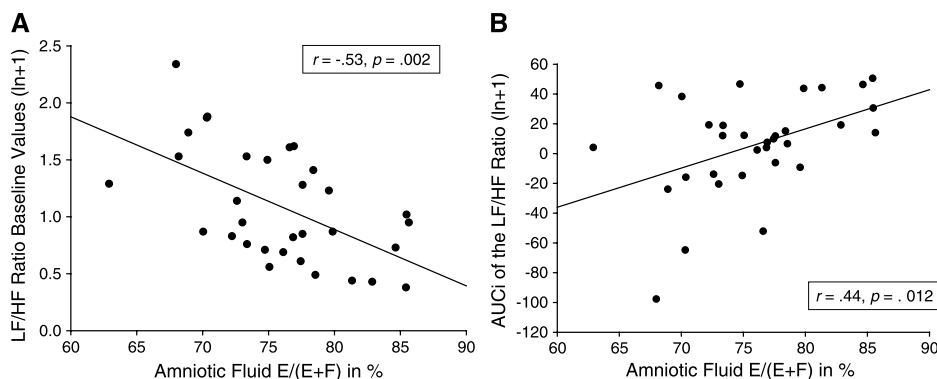


Figure 4. Bivariate correlations between the baseline levels of the ln transformed LF/HF ratio and the amniotic fluid E/(E + F) ratio in % (A) and between the AUCi (stress response) of the ln transformed LF/HF ratio and the amniotic fluid E/(E + F) ratio in % (B). HF = high-frequency component of heart rate variability; LF = low-frequency component of heart rate variability; E = cortisone; F = cortisol; AUCi = area under the curve with respect to increase.

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Surprisingly, sAA and HF increased significantly during the control condition while HR and LF/HF declined. HRV increases (i.e., respiratory sinus arrhythmia) during a control condition have been reported before (47) and may be a result of increased relaxation. Relaxation has been associated with increased HRV and decreased HR in third-trimester pregnant women (48). Also, increased sAA concentrations during relaxation have been found in nonpregnant participants (49) because sAA secretion is influenced by both sympathetic and parasympathetic activities (28). The women in our sample might likewise have been experiencing an increase in relaxation during the control condition and, therefore, an increase in parasympathetic activation. Our finding of a decrease in wakefulness across time in the control condition corroborates this interpretation.

The psychological stress measures in this study were hardly related to the physiological stress markers (i.e., maternal ANS and amniotic fluid parameters). This is a phenomenon often described in stress research (50,51). Interindividual differences in stress appraisal and in the tendency to respond to questionnaires in a socially desirable manner might explain this lacking association. An alternative explanation might involve the use of the MMSQ in the present study as a measure to assess the pregnant women's acute psychological stress response to amniocentesis. The MMSQ measures general mood states. The stress, fears, and worries of a pregnant woman regarding the well-being of her unborn child in an amniocentesis situation might not have been sufficiently captured this way thus, leading to a lack of associations with physiological stress measures. Future studies should take this factor into account. Also, excluding pregnant women with psychiatric diagnosis may have limited the variance in psychological stress responses and similarly contributed to this result.

Investigating the ANS during human pregnancy can further our understanding of how maternal stress may reach the fetus and initiate fetal programming effects. Evidence for an important role of the ANS in fetal programming comes from studies reporting a positive association between the blood pressure response of healthy pregnant women and shorter gestational length and low birth weight (52). In various epidemiological studies, low birth weight has been identified as a predictor of cardiovascular and metabolic disorders in adulthood even within the normal birth weight range (cf. Ref. (9)). Furthermore, children's systolic blood pressure response at 3 years was predicted by the ratio between E and F obtained from umbilical cord blood samples at delivery (53). The maternal ANS responses to stress may regulate the activity of the placental barrier enzyme 11 β -HSD2. In vitro, 11 β -HSD2 mRNA is down-regulated by NE and EPI (24), leading to the assumption that chronic activation of the maternal ANS due to stress may weaken 11 β -HSD2 activity and, as a result, lead to fetal F excess. Results from the current study reporting a negative association between LF and LF/HF baseline and amniotic E/(E + F) and simultaneously a positive association between LF and LF/HF baseline and amniotic F parallel results of impaired 11 β -HSD2 activity in chronically stressed pregnant rats (12,13). Increased baseline levels of stress markers, including LF/HF, have been found to reflect chronic stress exposure in

humans (54). However, our results still need to be viewed with caution and confirmed in future studies. In addition, chronic ANS activation may alter 11 β -HSD2 activity by reduced placental oxygen transfer. Reduced oxygen seems to impair 11 β -HSD2 (14). Correspondingly, prenatal anxiety (55) and psychological distress (56) have been associated with impaired placental blood flow, which, in turn results in reduced placental oxygen supplies (14).

The significant relationship between the acute LF/HF stress response and amniotic E/(E + F) may indicate that higher sympathovagal balance or, in other words, a greater shift to sympathetic activation caused by stress, induces an up-regulation of placental 11 β -HSD2 activity in humans and corresponds to findings in acute-stressed pregnant rats (12). Likewise, increased LF/HF and also LF stress responses in our study were associated with lower amniotic fluid F levels. This suggests that the acute maternal stress response may activate allostatic processes in the placenta, in agreement with the compensatory stress reactivity model of McEwen (57), to shield the fetus from the detrimental effects of F excess. Chronic stress, however, may weaken this protective shield.

Although correlations between maternal LF, LF/HF, and amniotic fluid F and E/(E + F) were found, thereby pointing toward an involvement of the maternal ANS in the regulation of fetoplacental 11 β -HSD2 activity, it is improbable that these ANS parameters are causally responsible for stress-induced changes in 11 β -HSD2. It is more likely that LF and LF/HF mirror the activity of other ANS variables known to impair the actions of the placental barrier enzyme, such as NE and EPI (24) or placental oxygen transfer (14). However, future studies need to investigate this hypothesis in more detail.

One of the main limitations of the current study is that we did not measure placental 11 β -HSD2 activity directly, which is technically and ethically not possible during ongoing human pregnancy. We measured amniotic fluid E/(E + F) as a marker of 11 β -HSD2 in the fetoplacental unit. The ratio between F and E obtained from umbilical venous cord blood after birth has been found to correlate positively with placental 11 β -HSD2 activity (58). However, it remains unclear to what degree amniotic fluid E/(E + F) at 15 to 17 weeks of gestation is a result of placental or fetal 11 β -HSD2 activity, but because the placenta has a larger capacity of metabolizing F than the fetus (22), a large fraction of the E/(E + F) ratio may reflect placental metabolism.

Obviously, it was also not possible to monitor the amniotic fluid parameters as the maternal stress response unfolded over time. Therefore, no clear statements can be made about the effect of the maternal ANS response on the fetal stress response. Moreover, although stress is capable of activating the adult ANS within seconds, the time it takes for the maternal ANS response to be measurable in the amniotic fluid is unclear. In pregnant rabbits and guinea pigs, continuous infusion of NE and EPI reduces placental blood flow causing fetal HR to decrease within 20 to 60 seconds after the infusion started (59). Likewise in humans, fetal HR decreases within 1 minute after a single injection of NE to the mother (60).

A further limitation concerns the rather advanced mean age of 37 years in our sample of pregnant women, which possibly

influenced the measurement of both psychological and physiological markers to a certain degree. However, the mean age of 37 years is not unusual for pregnant women undergoing amniocentesis because the risk of chromosomal abnormalities increases with advancing age. It also should be noted that the average age of maternity in Switzerland has risen from 30.8 years in 2007 to 31.48 years in 2011 (30).

Whether and to what degree our results are influenced by the activity of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which mainly catalyzes the opposite reaction as 11 β -HSD2, namely, the conversion of E to F, are unclear. 11 β -HSD1 is present in the decidua, the chorion, and the endothelium of the placental villous tissue and umbilical cord (61). However, activity has not been detected in the fetal membranes between 16 to 19 weeks of gestation (22,62). Moreover, 11 β -HSD2 activity strongly predominates 11 β -HSD1 activity in the human placenta (63).

The strengths of the study at hand include the examination of the psychobiological stress response of different psychological and ANS parameters in pregnant women confronted with a naturalistic stress situation and the comparison of these with amniotic fluid F, E, and E/(E + F). The findings indicate that the ANS and its potential association with 11 β -HSD2 in the fetoplacental unit is an important path for future research in understanding how maternal prenatal stress may reach the fetus.

The authors would like to express their gratitude and appreciation to the Swiss National Science Foundation for supporting this study.

The authors would also like to thank C. Breyman, G. Hebisch, M. Hodel C. Honegger, M. Kaufmann, R. C. Müller, and T. Roos for their support in the recruitment of the participants for this study. Furthermore, the authors would like to acknowledge the help of I. Gehrman, L. Gurzan, S. Kobelt, A. Kündig, S. Scherrer, and D. Thierstein for their continuous efforts in this project. A special thank you goes to Marina Haller for her assistance with the statistical analyses. Last but not least, the authors would like to express their deep gratitude to the mothers who volunteered to participate in this study.

Source of Funding and Conflicts of Interest: This study was supported by the Swiss National Science Foundation Grant (105314_120586) (to U.E. and R.Z.). The authors have no conflict of interest to declare.

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