Running title: SALIVARY ALPHA-AMYLASE DURING PREGNANCY

Salivary alpha-amylase during pregnancy: Diurnal course and associations with obstetric history, maternal demographics and mood

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Abstract

Diurnal patterns of salivary alpha amylase (sAA) in pregnant women have not previously been described. The current study employed ecological momentary assessment to examine the association between the diurnal sAA, obstetric history, maternal demographics, and mood during pregnancy. Saliva was self-collected by 83 pregnant women (89% White, age 25.3-43.0 years; mean gestational age 21.9 weeks, range 6-37 weeks; gravida 1-6) at home over three days. Results indicated that current pregnancy (gestational age and fetal sex) and maternal demographics were not related to diurnal sAA. In contrast, a history of previous miscarriage (Parameter = -.17; SE = .05; $p < .05$) was associated with an atypical diurnal pattern. Even after accounting for obstetric history, trait anxiety (Parameter = .16; SE = .04; $p < .001$) was associated with increased sAA over the day while chronic levels of fatigue (Parameter = -.06; SE = .03; $p < .05$) were associated with decreased sAA. In a separate model, we also tested the time varying covariation of sAA and mood. The effects of momentary mood were in contrast to those for trait mood. Both momentary depression (Parameter = .22; SE = .09; $p < .01$) and vigour/positive mood (Parameter = .12; SE = .04; $p < .001$) were associated with momentary increases in sAA while momentary anxiety and fatigue were not related to sAA. The findings suggest that basal sAA during pregnancy is sensitive to emotional arousal. Evaluating diurnal patterns of sAA holds promise for advancing understanding of how emotional arousal during pregnancy may affect fetal development.

Keywords: salivary alpha-amylase, psychological distress, pregnancy, stress, mood, obstetric history

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Recent advances in fetal science have generated interest in the psychobiology of stress during pregnancy (Entringer et al., 2010). There is now a sizable literature demonstrating that maternal psychological distress during pregnancy is associated with adverse developmental outcomes and this evidence has been interpreted as supporting the fetal programming hypothesis (e.g., Kinsella & Monk, 2009). In brief, the fetal programming hypothesis postulates that because the morphological and functional organization of a variety of biological systems are sensitive to environmental input during critical windows of prenatal exposure, the nature of such exposure permanently programs set points within those systems (Barker, 1998). Growing support for the fetal programming hypothesis has fueled the search for plausible biological mechanisms which could transduce the maternal psychological distress signal to the fetus and thereby alter the course of development.

Over the past decade, a growing body of literature suggests multiple pathways through which maternal experience may affect fetal development (Dunkel-Schetter, 2011). The focus of this work, however, has primarily targeted the psychobiological stress response and in particular the linkages between maternal cortisol and offspring developmental outcomes (e.g., Davis, Glynn, Waffarn, & Sandman, 2010). This relatively exclusive focus is surprising for at least two reasons. First, the majority of studies examining naturally occurring stressors during pregnancy have reported non-significant or low associations across various biological measures of cortisol, including amniotic fluid (Bergman, Glover, Sarkar, Abbott, & O'Connor, 2010), serum (Bergman, Sarkar, Glover, & O'Connor, 2010; Davis et al., 2010; Goedhart et al., 2010; Petraglia et al., 2001), and saliva (Davis et al., 2007; Davis & Sandman, 2010). Second, cortisol is only

one potentially important pathway through which maternal stress may impact the developing fetus. For example, cardiac autonomic change in utero-placental blood flow (i.e., vascular resistance) is associated with increased psychological distress among pregnant women (Teixeira, Fisk, & Glover, 1999), and adverse obstetric outcome, including pre-eclampsia and intrauterine growth restriction (Karsdorp et al., 1994; Van den Bergh, Mulder, Mennes, & Glover, 2005). Furthermore, it has been shown that normal variation in maternal anxiety is associated with changes in fetal blood distribution such that higher anxiety results in less blood flow to the fetal body in favour of blood flow to the brain (Sjöström, Valentin, Thelin, & Marsál, 1997). Such evidence suggests that maternal psychological distress may have important effects on fetal development via autonomic function.

Advances in biotechnology have enabled developmental scientists to estimate individual differences in the activity of the autonomic nervous system (ANS) via saliva. Salivary alpha-amylase (sAA) is an enzyme (protein) that is produced in the mouth by the salivary glands. Although production of salivary proteins is primarily associated with sympathetic stimulation, parasympathetic impulses are also involved (Proctor & Carpenter, 2007). Accordingly, stress-related changes in sAA levels have been proposed as a marker of ANS reactivity to stress (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; van Stegeren, Rohleder, Everaerd, & Wolf, 2006). In support of this proposal, levels of sAA are responsive to a variety of chemical, physical and psychological challenges (reviewed by (Nater & Rohleder, 2009). Furthermore, individuals with relatively higher levels of stress have higher levels of total sAA output over the course of a day (Nater et al., 2007). Like cortisol, sAA exhibits a distinctive diurnal pattern; however its' phase is opposite to that of salivary cortisol with levels decreasing sharply after waking and then increasing over

the remainder of the day (Nater et al., 2007; O'Donnell, Kammerer, O'Reilly, Taylor, & Glover, 2009; Rohleder, Nater, Schlotz, Ehlert, & Kirschbaum, 2004).

Recent studies (Adam & Granger, 2011; Adam, Till Hoyt & Granger, 2011; Nater et al., 2007) have challenged the notion that sAA is exclusively a marker of stress. The findings from this research demonstrate that, in addition to associations with stress, levels of sAA and positive mood covary over time. That is, levels of sAA increase in response to both increases in distress and positive mood. Adam and Granger (2011) proposed that sAA may be a marker of emotional arousal rather than distress per se. The potential for such non-specific effects requires further evaluation in order to clarify the role of sAA as a biomarker in stress research. Furthermore, the associations between sAA and mood in pregnant women require elaboration in order to understand their implications for fetal development.

Pregnancy-related change in sAA secretion has received little attention. Several small studies suggest that salivary flow rate and sAA levels are not affected by advancing gestation (D'Alessandro, Curbelo, Tumilasci, Tessler, & Houssay, 1989; Salvolini, Di Giorgio, Curatola, Mazzanti, & Fratto, 1998). We could find only one previous study reporting on the link between sAA and stress during pregnancy: it found that pregnancy attenuates increases in sAA in response to a laboratory stressor (Nierop et al., 2006). The diurnal course of sAA during pregnancy has not previously been described.

In the present study, we investigated patterns of diurnal changes in sAA among pregnant women. Our interest in sAA was motivated by the possibility that aspects of autonomic function may have direct or indirect effects on fetal development. Our specific aims were to determine: (1) the diurnal pattern of sAA secretion during pregnancy, (2) whether individual differences in obstetric history and characteristics of current pregnancy moderate the diurnal pattern, and (3)

whether positive or negative mood is associated with diurnal changes in sAA. To accomplish these aims, we collected detailed pregnancy and obstetric information and conducted an ecological momentary assessment study in which pregnant women collected saliva samples and reported their mood over the course of three days.

Methods

Participants

A subsample of 96 pregnant women between 6 and 37 weeks gestational age (GA), M = 21.9 weeks, SD = 8.7, who were enrolled in a study of nutrition during pregnancy (see www.ApronStudy.ca for further details) participated. Women were excluded if they reported any of the following: a) taking a steroid medication, b) smoking, c) consuming alcohol or drugs, d) recent dental work or tendency for oral bleeding (Granger et al., 2007), e) any known maternal or fetal medical complications (e.g., gestational diabetes, preeclampsia, fetal genetic anomalies), and g) illness during data collection (e.g., fever). Participants were mainly White (89%), well educated (72% with a university degree), and financially stable (54% earned more than $100,000/year; 9.6% earned less than $40,000/year). Half of the sample (50.6%) had not previously given birth and 33.7% were pregnant for the first time. Previous miscarriage was reported by 31%. We note that occurrence of the miscarriage was not medically confirmed. The observed rates of miscarriage were higher than the estimated 14 – 20% of miscarriage in clinically recognized pregnancies, however it is comparable to estimates from community-based assessment, where many cases remain unreported and therefore not medically confirmed (Everett, 1997). Of those who had given birth 9.4% had a preterm baby. Women were in their 1st trimester (n = 27), 2nd trimester (n = 30), and 3rd trimester (n = 26). Ethics approval for this study

was obtained from the Health Research Ethics Boards at the University of Calgary. Written consent was obtained from all women prior to enrolment in the study.

Materials and Procedure

Participants attended an individualized training session where they received instruction about using the personal digital assistant (PDA) data collection device and saliva collection. Body measurements were then collected by a trained assistant. Following Nater and colleagues (2007), saliva was collected using salivettes (Sarstedt Germany) which were placed under the tongue for 2 minutes. Women collected their own saliva at home over 3 consecutive days (excluding weekends) on the following schedule: upon waking, 30-45 minutes after waking, and semi-randomly about the anchor times of 1100h, 1600h, and 2000h. The semi-random signals occurred within 30 minutes following the anchor times. The exact time of these signals was therefore slightly different for each person. Each person received one signal within the 30 minute window for each of the sampling times. This semi-random signal was designed to reduce the possibility of changes in mood associated with anticipation of the signal received from the PDA. In order to facilitate adherence to the study protocol, the PDA was programmed to allow a 30 minute time window following the signal in which participants could respond after which data were considered missing.

Participants were instructed to turn on the PDA upon awakening, record their waking time, and collect a saliva sample. The PDA was programmed to ring 30-45 minutes later at which time participants collected their second saliva sample of the day. This procedure was used to assess the sAA awakening response while allowing individualized wake times. Each time the PDA rang, it first provided a unique code corresponding to a prelabelled saliva tube and asked participants to click “OK” when they had placed the saliva roll under their tongue. This was

designed to provide an exact time stamp for each saliva sample from the PDA record and to encourage adherence to the sampling protocol. The PDA administered the momentary mood questionnaire during the saliva collection. Participants also completed a standardized paper-and-pencil mood questionnaire (Profile of Mood States-Brief; McNair & Heuchert, 2003) that assessed individual differences in psychological distress.

Measures

Determinants of the diurnal course of sAA in pregnant women have not been previously described; therefore we included a broad set of measures to correspond with those assessed by Nater in colleagues (2007) in non-pregnant adults.

sAA assessment and determination. Participants were asked to refrain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing teeth in the 30 minutes prior to saliva collection and to report adherence to these guidelines (Granger et al., 2007). Samples were frozen (in home freezers) until a convenient return time could be arranged (usually within 1 week). Saliva samples were stored at -20 C until they were shipped frozen by overnight delivery to Salimetrics laboratories (State College, PA) for analysis.

Following Granger and colleagues (2007), all samples were assayed for sAA using commercially available kinetic reaction assays (Salimetrics, State College, PA) without modification to the manufacturers recommended protocols. The assay employs a chromagenic substrate, 2-chloro-p-nitrophenol linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 2 minute period) in absorbance at 405 nm. Results are computed in U/mL of sAA. Intra-assay variation (CV) computed for the mean of 30 replicate

tests was less than 7.5 percent. Inter-assay variation computed for the mean of average duplicates for 16 separate runs was less than 6 percent.

Pregnancy characteristics. Gestational age for the current pregnancy was determined from self-reported date of the last menstrual period. Sex of the fetus was determined by parent report after birth.

Obstetric history. Obstetric history, including number of previous pregnancies, number of spontaneous abortions, and number of preterm births were obtained by interview and confirmed by review of medical records.

Anthropometrics. All body measurements were taken following methodologies outlined in the Anthropometric Standardization Reference Manual (Lohman, Roche, & Martorell, 1988). Weight was obtained using a balance beam scale, measuring to the nearest 0.1kg. Height was measured to the nearest 0.1cm using a SECA 214 portable stadiometer. Lange Skinfold Calipers were used for skinfolds (bicep, tricep, subscapular, suprailiac, thigh) measured to the nearest 0.5mm. All skinfolds were grasped 1cm above the landmark, with calipers applied 1cm below the fingers (exactly on the landmark); with the exception of the suprailiac skinfold where the fold was made directly on the marked area, with calipers placed 1cm diagonally below the fold. Calipers were held in place for 4 seconds. Measurements were repeated 3 times on each site on the right hand side of the body; or until 3 skinfold readings for each site fell within 2mm (1mm for biceps). Research assistants were trained to reliability (inter-rater reliability coefficient of variation was less than 15% and intra-rater reliability coefficient of variation was less than 12%), and maintained reliability through periodic (every 3-4 months) reliability checks.

Individual (trait) mood. Individual differences in trait mood were assessed via a paper-and-pencil version of the Profile of Mood States-Brief (POMS-B), a 30 item multidimensional

measure of mood (McNair & Heuchert, 2003). The POMS-B allows the researcher to select between 'right now' and 'during the past week' as the time period of interest: we asked participants to report on the past week. The POMS-B assesses 7 mood dimensions, but in order to reduce the number of comparisons here we report on only anxiety, depression, fatigue, and vigour/positive mood. Internal consistency for these sub-scales is excellent, with coefficient alpha ranging between .86 and .93 (McNair & Heuchert, 2003).

Momentary mood. Momentary mood was measured using the Profile of Mood States-15 (POMS-15), which was designed for momentary assessment with PDAs (Cranford et al., 2006). The scale assessed 5 mood dimensions: anger, anxiety, depression, fatigue, and vigour/positive affect (in order to reduce the number of variables we do not report on anger here). Participants rated each item on a 5-point Likert scale from not at all to extremely, based on how they were feeling in the previous 30 minutes. Thus, for each saliva sample we had concurrent mood ratings on the 4 dimensions of interest. In two separate samples, Cranford and colleagues (Cranford et al., 2006) demonstrated that the POMS-15 had appropriate reliability to detect within-person change processes. Construct validity of the POMS-15 was also supported by demonstrating sensitivity to changes in mood among participants experiencing a major life stressor.

Statistical Procedures

Body density was calculated for all women using Formula 1 (Durnin & Womersley, 1974).

Formula 1 Body Density (kg/m3) = (1.1567 - 0.0717 x log of sum of skinfolds).

Fat mass was then calculated using pregnancy specific Formula 2 (van Raaij, Peek, Vermaat-Miedema, Schonk, & Hautvast, 1988), where W_{FM} is weight of fat mass,

Formula 2 $$W_{FM} = \frac{W_B}{100} \times \left\{ \frac{503.1}{D_B} - 458.9 \right\}$$

W_B is weight of body, and D_B is density of body (derived from Formula 1). Finally, percent body fat was calculated as a ratio of weight of fat mass to body weight.

Hierarchical linear modeling (HLM) (Raudenbush, Bryk, & Congdon, 2004) was used to fit a series of models. Multilevel equations were specified at three levels to account for the nested data structure (measurement moments nested within days and days nested within persons). As described by Nater and colleagues (2007) separate models were constructed to test the fixed effects of person (level-3) variables (obstetric history, maternal age and anthropometrics, gestational age, fetal sex, and trait mood) and time-varying (level-1) variables (momentary mood). sAA values were log transformed to correct skewness.

Following Singer and Willet (Singer & Willett, 2003), we constructed a discontinuous individual change model in which time was parameterized in three ways: 1) a dummy variable to capture the awakening response (1 = 30-45 min post waking; 0 = all other times), 2) time since waking to describe linear changes over the day and 3) time since waking squared to model the curvilinear shape of the diurnal trajectories over the course of the day. The model postulates that the diurnal patterns for sAA can be characterized by a growth trajectory that is 'interrupted' by the awakening response.[1] Data were analyzed with HLM 6.08 software (Raudenbush et al., 2004). Missing data were estimated using full information maximum likelihood. All HLM results reported here represent the final estimation of fixed effects with robust standard errors.

Results

Missing Data and Adherence

The 83 participants who completed the study collected a total of 1008 saliva samples (out of a possible 1245) for which sAA could be determined. Noncompleters did not differ from participants on any demographic variables. Of the missing samples, 40 were missing due to

insufficient quantity of saliva for conducting the assay, PDA failure (n = 11), participant was busy (n = 148), experimenter error (n = 15), and other reasons not specified (n = 23).

To estimate adherence to the protocol, self-reported wakeup time was compared to timing of the 30-45 min post-waking sample recorded by the PDA. If participants had responded immediately to the PDA signal, then all responses would have occurred within a 30 to 45 min window. Mean response times was 33.7 minutes (SD = 4.4). Ninety two percent of these samples were taken within the 30-45 minutes of waking. Samples outside the 30-45 minute window were not used to estimate the awaking response.

Descriptive Statistics.

Descriptive statistics for study variables are presented in Table 1. Significant overall mean differences (averaged over the 3 days) in sAA were observed between 1st and 3rd trimester women mid morning and between 2nd and 3rd trimester women in the evening. We note, however, that the overall pattern of results from the multilevel model (reported in the next section) did not confirm differences in the diurnal sAA trajectory based upon gestational age.

Individual differences in sAA patterns over the day

Preliminary analysis. The occurrence of exercising was rare (1.1% of samples), while consuming food or drink within 30 minutes of sample collection was more common (11% of samples). Preliminary multilevel models indicated that neither eating/drinking nor exercising were significantly associated with momentary changes in sAA. Accordingly, they were excluded from further analysis.

Average diurnal trajectory. Results for the unconditional growth curve model are shown in Table 2 (Model 1) and Figure 1. To facilitate interpretation, the log-transformed parameter estimate for the waking level is presented here using anti-logged values and the slope

parameters are reported as percentages of change over time. The average waking level of sAA was 13.21 U/ml. The awakening response resulted in a 41% decrease in sAA 34 minutes post waking ($p < .0001$). The average linear effect of time was a 9% increase in sAA for each hour ($p < .0001$). This linear increase was accompanied by an average 0.3 % decrease in the quadratic time parameter for each hour squared ($p < .05$).

Associations with fetal sex, gestational age, obstetric history, anthropometrics and maternal age. The average diurnal trajectory was not associated with fetal sex, occurrence of a prior preterm birth, gestational age, maternal age, or percent body fat. As shown in Table 2 (Model 2) and Figure 2, a history of previous miscarriage was associated with a flatter linear sAA increase over the day (11% per hour, $p < .05$), and greater than expected increases in sAA toward the end of the day (0.7% per hour squared) compared to women with no previous miscarriage. Gravida was independently (but marginally) associated with both the linear and quadratic terms. For each additional pregnancy the sAA increases over the day were 6% larger ($p = .06$) per hour compared to the average increase and the deceleration of this increase was 0.4 % larger ($p = .09$) per hour squared compared to the average curve. Neither gravida nor miscarriage was associated with average waking levels or the awakening response.

Associations with trait mood. Gravida and history of miscarriage and their interactions with the linear and quadratic terms were retained to control for their influence when testing the effects of individual differences in trait mood. Table 2 (Model 3) shows the results for trait mood. Depression and vigour/positive mood were not associated with the average sAA trajectory. Trait anxiety was associated with larger sAA increases (17% per hour, $p < .001$) over the day (Figure 3) and a more pronounced deceleration (1% per hour squared, $p < .001$) toward the end of the day. The effects of ongoing fatigue were opposite that of trait anxiety. Fatigue was

associated with less sAA increase over the day (6% per hour, $p < .05$; see Figure 4) and marginally less curvature (0.4% per hour squared, $p = .08$) in the evening trajectory. Neither anxiety nor fatigue was associated with average waking levels or the awakening response.

Time-varying associations between sAA and momentary mood

The analysis of mood in the previous section examines how characteristics of the person (i.e., trait mood) affect the diurnal sAA pattern. To determine whether mood and sAA covary within persons over time we constructed a model in which we included momentary measures of anxiety, depression, fatigue and vigour/positive mood in the level 1 submodel of the multilevel model. As shown in Table 3, the pattern of associations between psychological distress and sAA for the time-varying model was different from that observed for the individual differences model. Momentary depression and vigour/positive mood were positively associated with sAA while momentary anxiety and fatigue were unrelated. For each one unit increase in momentary depression there was a corresponding 25% increase over average levels of sAA ($p < .01$) while for every one unit increase in momentary vigour/positive mood there was a corresponding 13% increase in sAA ($p < .005$).

Discussion

This study is the first to report on the diurnal pattern of sAA among pregnant women. Individual differences in current pregnancy (fetal sex, GA) and maternal characteristics (age and anthropometrics) were not related to sAA, however obstetric history was associated with changes in the diurnal pattern. Previous pregnancy experience, and especially previous unsuccessful pregnancy, was associated with an altered sAA pattern compared to women with no such history. Even after accounting for obstetric factors, individual differences in average levels of anxiety and fatigue moderated the diurnal patterns such that anxiety was associated with overall

increases in sAA while fatigue was associated with overall decreases. These effects of chronic mood were in contrast to the momentary mood effects. Within an individual, levels of sAA increased when either depression or positive mood increased. These findings suggest that basal sAA secretion during pregnancy is sensitive to emotional arousal.

The contrasts between the momentary and chronic mood effects are intriguing. We note first that it is not possible to directly compare the momentary to the chronic mood effects. The momentary effects describe the time varying covariation between mood and sAA and answer the question "does an individual's sAA change in concert with changes in mood?" The chronic mood effects, in contrast, describe individual differences in mood and sAA and answer the question "Are individuals high (or low) in sAA also high (or low) in mood?" These are rather different questions. Nevertheless, it is interesting to note that the grouping of mood effects related to individual differences (anxiety and fatigue) form a physiological arousal dimension while the mood effects associated with momentary changes in sAA are more related to psychic arousal. It may be that ongoing levels of physiological arousal have an effect on basal sAA secretion while more transient changes in psychic arousal do not. In contrast, moment-to-moment changes in psychic arousal may result in short-term perturbations from tonic levels but have less effect on overall basal levels.

Overall, the pattern of associations between mood and sAA, suggests that sAA is a non-specific physiological marker of mood. These findings support the "arousal hypothesis" proposed by Adam and Granger (2011) to account for the fact that both positive and negative aspects of mood are associated with sAA increases. The current study replicates two previous studies (Adam & Granger, 2011; Adam, Till Hoyt, & Granger, 2011) suggesting that level of emotional arousal rather than emotional valence is associated with sAA increases. Furthermore,

our novel findings regarding the inverse association between fatigue and sAA support the arousal hypothesis by demonstrating that levels of sAA are associated with both arousal and low energy (i.e., under arousal).

The finding that individual differences in current pregnancy, including GA and fetal sex, were not associated with changes in the diurnal pattern of sAA is consistent with previous studies which found no differences in single measures of sAA at different stages of pregnancy (D'Alessandro et al., 1989; Laine et al., 1988; Salvolini et al., 1998). We note that individual differences in gestation did result in mean differences in sAA levels (see Table 1) for mid morning and evening samples. These differences, however, did not affect the overall diurnal pattern, as revealed by the multilevel model. These findings are in contrast to the systematic increases in cortisol that typically occur during pregnancy (Mastorakos & Ilias, 2003; Sandman et al., 2006) and to the effects of fetal sex on diurnal cortisol in late pregnancy (DiPietro, Costigan, Kivlighan, Chen, & Laudenslager, 2011). It should be noted, however, that GA in the current study was assessed by self-report of last menstrual period and not confirmed by ultrasound. Furthermore, studies that prospectively follow women throughout pregnancy are needed to provide a stronger test of the effects of GA.

Previous pregnancy experience, and in particular spontaneous abortion, was associated with an altered diurnal sAA pattern. Early in the day, patterns of sAA were similar for all women but women with a history of previous miscarriage had significantly flatter sAA trajectories through the mid portion of the day and they failed to display the deceleration of sAA increase that is typically observed in the evenings. These findings may suggest long-term alteration in patterns of sAA secretion that are associated with pregnancy experience. Adaptation to pregnancy includes profound changes in physiology, some of which may persist long-term. For

example, parity has a well-documented inverse association with breast cancer risk later in life (Collaborative Group on Hormonal Factors in Breast Cancer, 2002). As we did not assess women in postpregnancy we cannot determine whether alteration of the diurnal sAA pattern is evident in women with a history of miscarriage when they are not pregnant. Further research is required to determine whether the observed changes in sAA patterns are general physiological adaptations, adaptations that occur only during pregnancy, or a chance observation within our sample. In addition, it will be important to determine whether changes in sAA associated with previous miscarriage are associated with changes in emotional arousal and whether emotional arousal mediates the association between miscarriage and sAA. Women with a history of miscarriage tend to also display higher levels of anxiety and depression during a subsequent pregnancy (Blackmore et al., 2011) therefore the effects of miscarriage may be related to increases in psychological distress. It should be noted, however, that the effects for miscarriage remained significant (although they were reduced) even when the mood variables were included (Table 2, Model 3) indicating that the effect of miscarriage was independent of mood.

The findings have potential implication for understanding the effects of maternal mood on fetal development. A growing number of studies have demonstrated that, even after adjusting for many possible confounders, such as socioeconomic status, maternal age, birth weight, gestational age, and biomedical risks during pregnancy, maternal psychological distress during pregnancy predicts offspring emotional dysregulation (O'Connor, Heron, Golding, & Glover, 2003), cognitive ability and fearfulness (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007), motor development (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), and increases in ‘difficult’ temperament (Austin, Hadzi-Pavlovica, Leader, Saint, & Parker, 2005).

To what extent such effects are specific to emotional distress or more generally related to emotional arousal has not been assessed.

Studies that combine assessment of cortisol and sAA are needed to provide further insight into the effects of acute and chronic maternal mood on fetal development. Monk and colleagues (Monk et al., 2011; Monk et al., 2004), for example, have suggested that stress-related biological signals to the fetus may operate in combination such that maternal cortisol may potentiate fetal hyper-reactivity to stress and maternal autonomic arousal may then stimulate further alteration to fetal development, such as increases in heart rate. Alternately, emotional arousal (as indexed by sAA), may alter the potency of other signals, cortisol for example, through increases in autonomic arousal. Optimum functioning after exposure to stressful situations requires coordination between stress response systems (Ali & Pruessner, 2011). Coherence (and non-coherence) among psychophysiological and biological systems may be an important factor in determining the effects of the individual markers on the developing fetus.

Ecological momentary assessment, providing three days of diurnal sAA with up to 15 measurements per woman, and the simultaneous assessment of mood at the momentary (state) and individual (trait) levels, is a significant strength of this study. Nevertheless, several limitations require consideration. First, the time varying variables were measured concurrently, which raises the problem of reciprocal causation. That is, changes in sAA may be responsible for changes in mood. However, experimental studies showing that administration of the beta adrenergic antagonist propranolol leads to decreases in sAA during a stressful procedure (van Stegeren et al., 2006) argue against this interpretation. Second, the sample represents a relatively healthy population of well-educated, employed, mature, and Caucasian women with uncomplicated singleton pregnancies. Caution should be exercised in extending these findings to

socially disadvantaged or medically complicated pregnancies. More broadly, the limitations of this study include a modest sample size, collection of data at only one point in pregnancy, and self-report for many of the pregnancy variables (e.g., gestational age and history of previous miscarriage).

In summary, we found that levels of sAA over the course of 3 days were associated with mood and obstetric history but unrelated to individual differences in GA, fetal sex, maternal age, and anthropometrics. These findings replicate previous studies demonstrating a link between mood and the diurnal pattern of sAA and extend the overall pattern of results to pregnant women. The current findings support the use of sAA as a measure of emotional arousal, as opposed to a measure of psychological distress per se. Although sAA was a non-specific marker of emotional arousal in this study, it remains a promising tool for understanding how the fetus incorporates the experiences of its mother into its own development, especially when measurement of sAA is combined with other potential mediators, such as cortisol. Accordingly, studies are needed to determine whether the effects of maternal emotional arousal, as indexed by sAA, have differential implications for fetuses exposed to high and low levels of maternal cortisol.

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Footnote

[1]We also tested a contrasting model that postulates a discontinuity in slope, not elevation, with an inflection point coinciding with the sAA awakening response. This model assumes that the diurnal course of sAA is best represented by two (or more) slope functions with an inflection point at 30 minutes post waking. The model we tested included three time parameters: one that was centered at waking to capture the sAA awakening response, and two additional time parameters (Time, and $Time^2$) that were centered at the inflection point (30 minutes post waking) to capture the trajectory over the remainder of the day. The results of this model were comparable to the model we report: that is, there was a significant negative slope over the first 30 minutes of the day, a significant positive slope over the remainder of the day, and a significant decline in this slope toward the end of the day. Our decision to focus on the 'level' model rather than the 'slope' model was based on the superior ability of the level model to capture the actual shape of the observed data.

Table 1

Means (standard deviations) for Study Variables by Trimester.

	1st Trimester (n = 27)	2nd Trimester (n = 30)	3rd Trimester (n = 26)	F
sAA in U/ml				
Waking	26.06 (26.96)	17.86 (20.61)	22.26 (30.63)	1.49
Waking + 34 min	14.73 (17.54)	10.77 (10.92)	10.19 (12.80)	1.99
Mid morning	34.17 (30.74)	29.96 (29.57)	21.36 (22.71)	4.21* (1,3)
Mid afternoon	36.01 (27.38)	34.88 (37.32)	27.33 (27.04)	1.51
Evening	35.22 (29.13)	45.87 (41.92)	26.36 (30.72)	6.16** (2,3)
Momentary Mood				
Anxiety	.31 (.44)	.38 (.49)	.32 (.43)	2.44
Depression	.16 (.35)	.18 (.35)	.14 (.37)	1.38
Fatigue	.88 (.91)	.85 (.94)	.99 (.95)	2.04
Vigour/Positive	1.04 (.75)	.92 (.78)	.97 (.82)	1.79
Trait Mood				
Anxiety	.65 (.41)	.61 (.45)	.58 (.57)	.13
Depression	.41 (.32)	.41 (.48)	.33 (.42)	.28
Fatigue	1.4 (.95)	1.2 (.82)	1.5 (.68)	.87
Vigour/Positive	1.2 (.77)	1.2 (.79)	1.01 (.50)	1.05
Trait Mood				
Anxiety	.65 (.41)	.61 (.45)	.58 (.57)	.13
Depression	.41 (.32)	.41 (.48)	.33 (.42)	.28
Fatigue	1.4 (.95)	1.2 (.82)	1.5 (.68)	.87
Vigour/Positive	1.2 (.77)	1.2 (.79)	1.01 (.50)	1.05
Maternal Characteristics				
Age	33.4 (4.1)	32.6 (4.3)	31.2 (4.6)	1.95
Percent Body Fat	30.7 (4.2)	32.9 (4.4)	32.2 (3.8)	1.74
		Percentage		
Obstetric History				
Preterm birth	7.7	3.3	11.5	
Miscarriage	33.3	26.7	34.6	
Current Pregnancy				
Primigravida	22.2	33.3	46.2	
Female fetus	46.2	48.3	54.5	

Note: * $p < .05$, ** $p < .01$. The numbers in brackets following significant F tests indicate trimester comparisons which were statistically significant.

Table 2

Multilevel Models for Average Diurnal sAA Trajectories (Model 1), Effects of Current Pregnancy and Obstetric History (Model 2), and Effects of Individual Differences in (Trait) Mood (Model 3)

	Model 1			Model 2			Model 3		
Fixed Effects	Estimate	*SE*	*p*	Estimate	*SE*	*p*	Estimate	*SE*	*p*
Momentary (level 1) Effects									
WAKING Levels	2.58	.102	< .001	2.40	.157	<.001	2.58	.099	<.001
sAA-AR	**-.532**	**.090**	**<.001**	**-.421**	**.125**	**.002**	**-.535**	**.088**	**<.001**
TIME	**.086**	**.023**	**<.001**	**.075**	**.030**	**.01**	**.060**	**.025**	**.02**
$TIME^2$	**-.003**	**.001**	**.05**	-.002	.002	.30	-.001	.002	.45
Moderator (level 3) Effects									
For WAKING Levels									
Gravida				.049	.135	.72			
Miscarriage				.237	.167	.16			
Anxiety							-.271	.254	.29
Depression							.438	.318	.17
Fatigue							.011	.131	.93
Vigour/positive mood							.114	.134	.40
For sAA-AR									
Gravida				-.005	.112	.96			
Miscarriage				-.211	.221	.34			
Anxiety							.205	.254	.42
Depression							.239	.253	.35
Fatigue							-.228	.140	.13
Vigour/positive mood							-.114	.123	.36
For TIME									

Gravida	.060	.030	.06	**.057**	**.024**	**.02**
Miscarriage	**-.117**	**.054**	**.03**	**-.082**	**.037**	**.02**
Anxiety				**.156**	**.043**	**.001**
Depression				-.012	.05	.81
Fatigue				**-.063**	**.032**	**.05**
Vigour/positive mood				-.005	.027	.87
For $TIME^2$						
Gravida	-.004	.002	.09	-.003	.002	.06
Miscarriage	**.007**	**.003**	**.04**	**.005**	**.003**	**.05**
Anxiety				**-.011**	**.003**	**.001**
Depression				.002	.003	.64
Fatigue				.004	.002	.08
Vigour/positive mood				-.001	.002	.80

Note: Words in capital letters indicate time-varying (Level 1) variables. Bolded values indicate $p < .05$. sAA-AR = salivary alpha-amylase awakening response.

Table 3

Multilevel Model for Momentary Mood Effects on sAA

Fixed Effects	Estimate	*SE*	t-ratio	Interpretation
Waking levels	2.16	.156	13.81***	sAA waking level = 8.7 U/ml
Awakening response	-.329	.105	3.11**	28% decrease in sAA 34 minutes post waking
Time	.136	.029	4.67***	14.6% increase in sAA per hour
$Time^2$	-.005	.002	3.15**	0.5% decrease in sAA per hour squared
Anxiety	-.022	.075	.29	n.s.
Depression	.225	.091	2.46**	25% increase in sAA per unit increase in depression
Fatigue	-.019	.043	.45	n.s.
Vigour/Positive mood	.124	.048	2.57**	13% increase in sAA per unit increase in positive mood

Note: * $p < .05$, ** $p < .01$, *** $p < .001$. U/ml = units per milliliter.

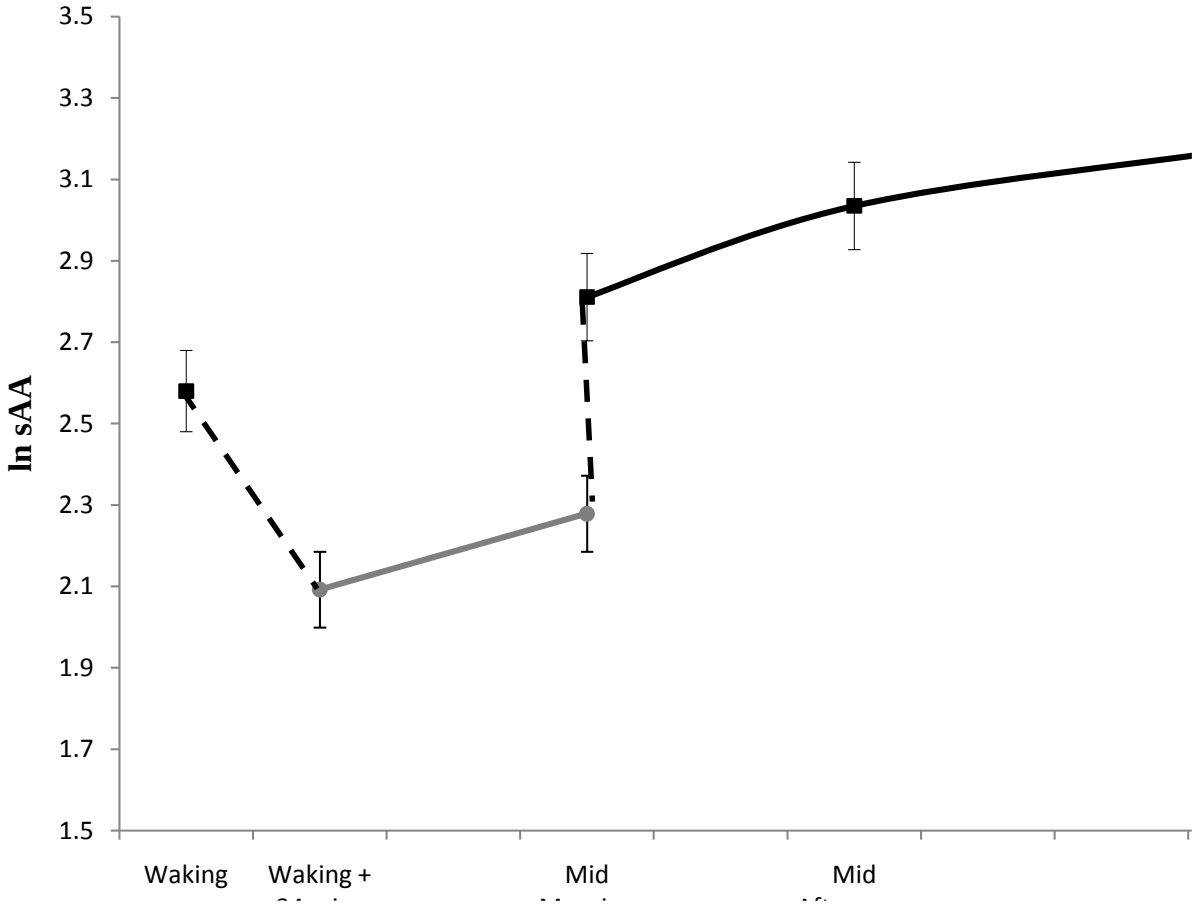

Figure 1. Average population diurnal sAA trajectory. Error bars are standard errors of the mean. The dashed portion of the line indicates a shift in trajectory associated with the awakening response. The gray portion of the line depicts the awakening response trajectory while the black portion depicts the trajectory over the remainder of the day.

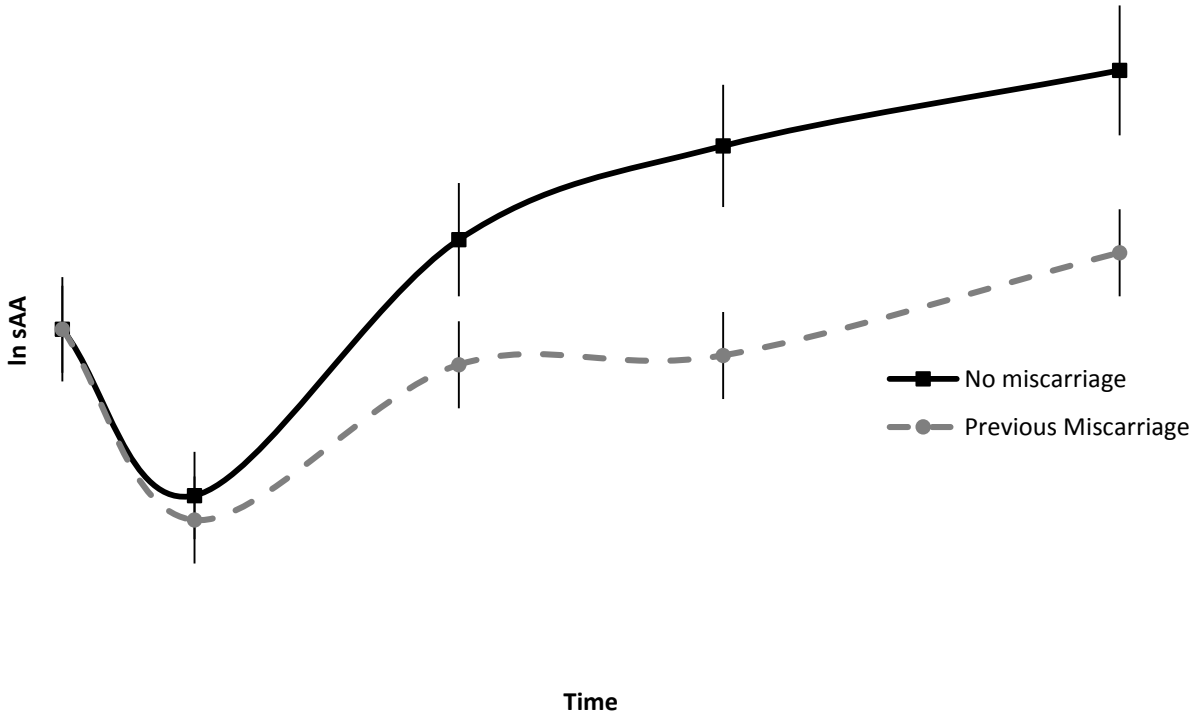

Figure 2. Effect of previous spontaneous abortion on diurnal sAA trajectory during current pregnancy. Error bars are 95% confidence intervals.

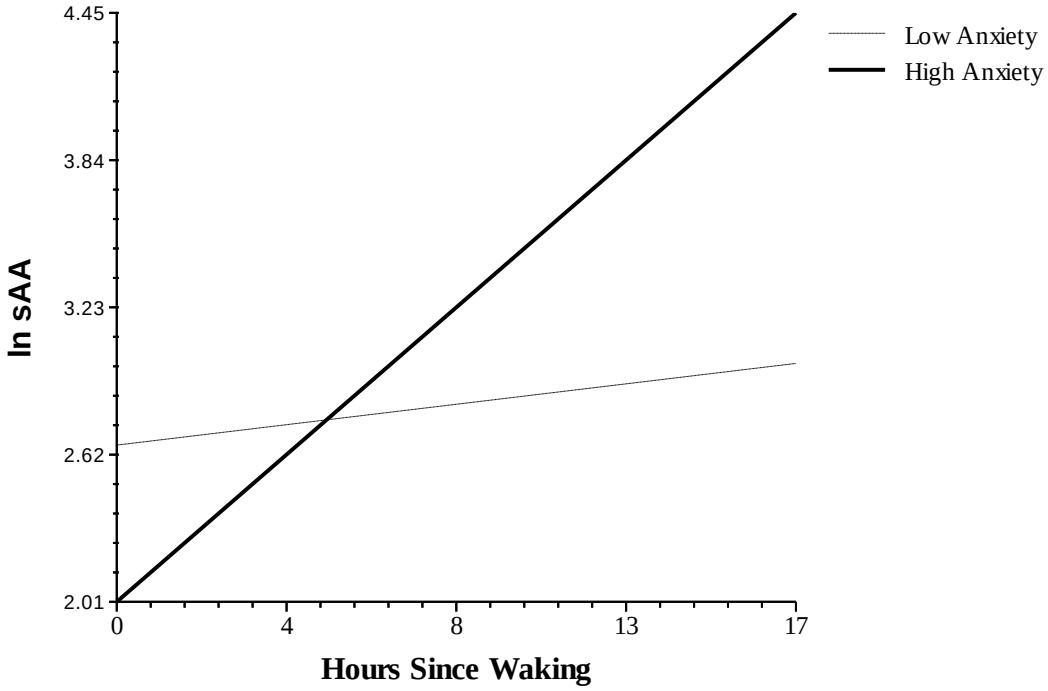

Figure 3. Linear effect of individual differences in trait anxiety on sAA trajectory over the day. Low anxiety = 25th percentile; high anxiety = 75th percentile

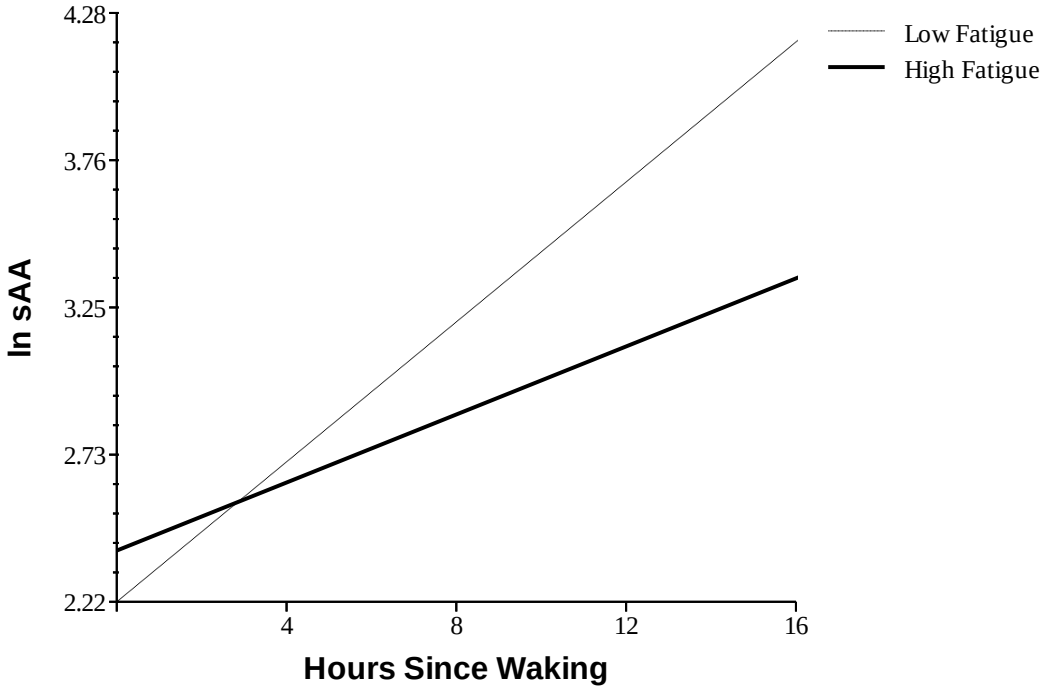

Figure 4. Linear effect of individual differences in fatigue on sAA trajectory over the day. Low fatigue = 25th percentile; high fatigue = 75th percentile