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Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: Comparison to salivary cortisol

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Abstract

Maternal stress during pregnancy is associated with negative maternal/child outcomes. One potential biomarker of the maternal stress response is cortisol, a product of activity of the hypothalamic-pituitary-adrenal axis. This study evaluated cortisol levels in hair throughout pregnancy as a marker of total cortisol release. Cortisol levels in hair have been shown to be easily quantifiable and may be representative of total cortisol release more than single saliva or serum measures. Hair cortisol provides a simple way to monitor total cortisol release over an extended period of time. Hair cortisol levels were determined from each trimester (15, 26 and 36 wks gestation) and 3 months postpartum. Hair cortisol levels were compared to diurnal salivary cortisol collected over 3 days (3 times/day) at 14, 18, 23, 29, and 34 wks gestational age and 6 wks postpartum from 21 pregnant women. Both salivary and hair cortisol levels rose during pregnancy as expected. Hair cortisol and diurnal salivary cortisol area under the curve with respect to ground (AUCg) were also correlated throughout pregnancy. Levels of cortisol in hair are a valid and useful tool to measure long-term cortisol activity. Hair cortisol avoids methodological problems associated with collection other cortisol measures such as plasma, urine, or saliva and is a reliable metric of HPA activity throughout pregnancy reflecting total cortisol release over an extended period.

Keywords

hypothalamic pituitary adrenal axis; stress biomarkers; thrifty phenotype; early programming

1. Introduction

Maternal stress negatively affects pregnancy outcomes and subsequent child development [1-3], but the mechanism by which this occurs is not clear. Cortisol, the hormonal product of activation of the hypothalamic-pituitary-adrenal (HPA) axis, has been suggested as a potential mechanism linking maternal stress and perinatal outcomes [4]. Recently, long-term estimates of prenatal HPA activity of the mother have been measured in hair collected in the

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postpartum period providing a novel retrospective means of assessing stress during pregnancy.

Abnormal patterns of maternal cortisol during pregnancy have been associated with negative perinatal outcomes including miscarriage [5], increased fetal activity [4], premature birth and decreased birth weight [4]. Elevated maternal stress and consequently, cortisol [6], negatively affect long-term HPA-axis regulation of the offspring [3, 7, 8] by reorganizing the offspring's HPA-axis. Dysfunction of peripheral aspects of the HPA-axis and increases in absolute cortisol has been correlated with maternal psychosocial reports of stress, though the results are inconsistent [9, 10]. This suggests that during pregnancy women may have altered physiological stress reactivity with potential consequences for infant outcome. Thus, maternal cortisol is a candidate physiological mediator between prenatal maternal stress and adverse outcome for the offspring.

Cortisol can be measured in a variety of ways. Many studies include single blood and/or saliva samples and, while useful, they are only snapshots of HPA activity. In addition, there is great individual variability of baseline cortisol levels between and within subjects due to circadian rhythms and system fluctuations based on homeostatic regulation [11]. Thus, cortisol level measured in saliva, even repeatedly, may not be the most informative measure to use to evaluate *overall or long term* HPA activity during the prenatal period. A measure integrating cortisol over a longer time frame may be more useful in quantifying the early perinatal environment of both the mother and her offspring.

Hair cortisol is a reliable measure of overall HPA activity in humans [12]. Cortisol measured from hair collected closest to the scalp estimates cortisol production retrospectively for up to 6 months [13]. It is unclear how cortisol becomes incorporated into the hair shaft, however, cortisol has been determined to be synthesized by the pilosebaceous unit located in the hair follicles in response to adrenocorticotrophic hormone [14]. Unbound cortisol may also diffuse from capillaries into the cells of the hair follicle and become deposited within the hair shaft as well [15]. Hair typically grows at a regular rate and cortisol levels in hair are easily measured by standard cortisol assay techniques [12, 13, 16, 17]. Hair and salivary cortisol are highly correlated in non-human primates [16] and significantly increase in response to an extended environmental challenge (Fairbanks, Jorgenson, Bailey, Breidenthal, Grzwy, & Laudenslager, submitted). Thus, hair cortisol has the potential to serve as an integrated measure of HPA activity over an extended period of time of up to 3-6 months.

Hair cortisol has been used to evaluate environmental stress in humans. For example, hair cortisol levels in hospitalized infants are increased compared to their healthy non-hospitalized counterparts. Hair cortisol increased for each additional day an infant was ventilated [17]. Hair cortisol is elevated during the third trimester of pregnancy [13] and is higher in association with stress during the late first trimester of pregnancy [18]. This is the first study to directly compare salivary cortisol and hair cortisol longitudinally from early pregnancy through the postpartum period. The goal of the present study is to compare hair cortisol with diurnal salivary cortisol *throughout* pregnancy and in the first three months postnatally.

2. Methods and Materials

2.1 Sample

Twenty one non-smoking pregnant women (ages 18-45), less than 17 weeks gestational age and experiencing non-complicated pregnancies were recruited to participate in the study. The primary exclusion criteria were use of any medications (other than prenatal vitamins) at time of enrollment or having bleached hair or using high-level peroxide products on their

hair. All research subjects provided informed consent under the Colorado Multiple Institutional Review Board.

2.2 Salivary cortisol collection and analysis

Saliva was collected using specially cut filter paper (Whatman Grade 42) contained in booklets for saliva collection as previously described [19, 20]. Saliva was collected on three days at each time point, *once* during the first trimester (14.0 ± 1.9 weeks gestation), *twice* during the second trimester (18.3 ± 0.2 and 23.3 ± 0.4 weeks gestation), *twice* in the third trimester (29.4 ± 0.7 and 33.9 ± 0.1 weeks gestation) and *once* in the postpartum period (6.6 ± 0.3 weeks postpartum). Thus six sets of three days of sampling (three samples/day) were collected over the course of their pregnancy and the postpartum period. In brief, collection filters were assembled in a small booklet that contained three filters/day and labeled for collection at 30 minutes after waking, before lunch, and +10 hours after waking. Sampling times were recorded on each booklet at the time of sample collection. This sampling schedule avoids most problems associated with food intake on salivary cortisol levels. Tooth brushing was not allowed prior to sampling and solids and fluids were withheld for at least 20 min prior to sampling. Filters were separated from each other in the collection booklet by waxed weigh paper to prevent cross contamination. Color tabs on the waxed paper dividers matched color codes on the cover of the booklet as well as the filter paper and ensured that the correct filter in the booklet was properly wetted. Saliva collection was taught via personal instruction as well as a DVD recording. This at-home collection method using filter paper booklets has previously been used and shown reliable results in a variety of populations [21-24].

Filters were carefully cut and extracted in 0.25 ml of assay buffer as previously described in detail [20]. In brief, the buffer containing the cut filters was shaken overnight in microcentrifuge tubes. Extraction buffer (25 μ l) was added in duplicate to the appropriate wells of the assay plate. Extraction dilutes the saliva approximately 1:5 which is determined for each lot of filter paper and corrected in the final levels reported. Cortisol concentration in the extraction buffer was determined using a commercial expanded range high sensitivity EIA kit (No. 1-3002/1-3012, Salimetrics, LLC) that detects cortisol levels in the range of 0.003 – 3.0 μ g/dl (0.083 – 82.77 nmol/L). Standard curves were fit by a weighted regression analysis using commercial software (Gen 5) for the ELISA plate reader (BioTek). After taking dilution into consideration, the detection limit is 0.019 μ g/dl (0.52 nmol/L). This kit shows minimal cross reactivity (4% or less) with other steroids present in the saliva. Controls run on every plate, for determination of inter-assay coefficients variability, are less than 7.5% for high and low control levels. Intra-assay coefficients of variation for duplicate determinations are less than 3%. This procedure has been cross validated with outside laboratories with excellent correspondence to both DELFIA and mass spectrographic procedures ($r^2 = 0.85$). Reported levels are within 10-15% of whole saliva reflecting losses associated with extraction.

2.3 Hair cortisol collection and cortisol analysis

Hair was collected from the same posterior vertex region on the head at an average of 15.2 ± 0.5 , 25.8 ± 1.0 and 35.5 ± 0.7 weeks gestation (mean $\pm$ std) and 11.7 ± 2.2 weeks postpartum. Subjects made appointments with a hair stylist with whom we made special arrangements. Hair was collected from a 1 cm² patch using scissors as close to the scalp as possible as recommended by the Society of Hair Testing [25]. The hair samples were wrapped in aluminum foil for protection and storage as previously described [26]. New growth that emerged at the site between each trimester allowed us to determine each individual's hair growth which averaged 3.1 cm per trimester. Thus, we were able to

estimate a women's first trimester hair growth for cutting the first hair sample to contain only hair grown in the previous 3 months.

At the time of the third trimester sample, an adjacent full length sample was cut as well as the new growth area to permit retrospective comparison from the full length hair to samples collected at the end of the 1st, 2nd and 3rd trimester. However, due to inadequate sampling we were only able to obtain sufficient hair samples from 14 of the 21 women for this additional retrospective 3rd trimester sample. This retrospective hair sample analysis was compared with the prospective growth segments previously collected and cut into equal segments represented by the new growth as determined from the second and third samples. Hair was washed twice in isopropanol and dried for 4 days, as previously described [13, 16]. Hair was ground using a Retsch ball mill for 10 min at 25 Hz. The powdered hair (50 mg) was weighed in glass tubes and extracted in 1ml of HPLC grade methanol at room temperature for 24 hours with slow rotation. Following extraction, the solution was transferred to a 2ml microcentrifuge tube and spun for 120s in a microcentrifuge at approximately 20,000g. Supernatant (600µl) was removed, placed into a new microcentrifuge tube and dried at 38°C under a stream of nitrogen for 30 minutes. Extracts were reconstituted with 400µl of assay buffer and cortisol levels determined using a commercial high sensitivity EIA kit (Salimetrics, LLC) according to manufacturer's directions. An internal control consisting of a large pool of previously ground mixed hair was extracted and run on each plate for determination of inter-assay coefficients of variation. Intra-assay coefficient of variation (CV) is less than 9% and inter assay CV is less than 5%.

2.4 Data analysis

Salivary cortisol levels at each time were determined based on the means of the three days for each sampling time. These were further summarized as the area under the curve with respect to ground (AUCg) [27]. AUCg was used as the best representation of total cortisol released across the day to correspond to hair cortisol levels. The area under the diurnal curve more closely represents cortisol values contributing the levels in the hair an integrated measure of cortisol level over time [13, 28, 29]. Both salivary cortisol AUCg early and late in the second and third trimester were averaged to create an average for each trimester. Pearson's correlations were used to examine the relationship between hair cortisol levels and AUCg cortisol levels as well as maternal psychosocial stress. A one-way repeated measures analysis of variance (One Way RM ANOVA) was used to examine the pattern of cortisol levels in both hair and saliva and perceived stress across pregnancy and the postpartum period. All statistical analyses were performed using commercial software (SPSS 18; SPSS, Inc. Chicago, IL).

3. Results

3.1 Sample characteristics

The mean age ($\pm$ standard deviation) of mothers in the study was 30 ± 1 years. All other maternal demographics, including age range, race, education, employment and marital status are included in Table 1.

3.2 Pattern of cortisol levels across pregnancy in saliva and hair

Self reported times of daily collection saliva times across pregnancy were not significantly different: wake +30 min ($F(3,20) = 0.365$; $p = 0.778$; RM ANOVA), lunch ($F(3,20) = 1.533$, $p = 0.216$; RM ANOVA) and wake+10 hr ($F(3,20) = 2.371$, $p = 0.08$; RM ANOVA). See Table 2 for saliva collection time mean and standard deviation at each phase during pregnancy. There was a significant effect of trimester on salivary cortisol AUCg ($F(3,20) = 14.5$,

$p < 0.001$; One Way RM ANOVA; Figure 1A). Post-hoc tests revealed that salivary AUCg cortisol were lower in the first trimester relative to the second ($t = 2.5$, $p = 0.02$; Holm-Sidak) and third trimesters ($t = 3.5$, $p < 0.001$; Holm-Sidak, Figure 1A). In addition, the postpartum period AUCg was lower than the first ($t = 2.4$, $p = 0.02$; Holm-Sidak), second ($t = 4.9$, $p < 0.001$; Holm-Sidak) and third trimesters ($t = 6.09$, $p < 0.001$; Holm-Sidak). No other differences were detected. Figure 2A indicates mean and standard deviation for salivary cortisol levels at each phase during pregnancy. Raw salivary cortisol levels significantly differed during pregnancy (Two-way RM ANOVA; trimester, $F(3,20) = 6.9$, $p < 0.001$; time, $F(3,20) = 50.1$, $p < 0.001$; interaction, $F(3,20) = 3.2$, $p = 0.007$; Figure 2A). Post-hoc tests revealed first trimester salivary cortisol levels at wake+30 min differed from both lunch ($t = 2.8$, $p = 0.007$) and wake+10 hour time ($t = 3.8$, $p < 0.001$), as well as second trimester levels at wake+30 min relative to lunch ($t = 7.4$, $p < 0.001$) and wake+10hr ($t = 7.6$, $p < 0.001$), third trimester at wake+30 min vs. lunch ($t = 4.814$, $p < 0.001$) and wake+10 hr ($t = 6.369$, $p < 0.001$) and postpartum wake+30 min vs. lunch ($t = 3.234$, $p = 0.002$) and wake+10 hr ($t = 3.739$, $p < 0.001$), demonstrating the expected diurnal decline. Post-hoc tests also revealed differences in the wake+30 min collection between first and second trimester ($t = 4.272$, $p < 0.001$), first and third trimester ($t = 3.271$, $p = 0.001$), second trimester and postpartum period ($t = 4.943$, $p < 0.001$) and the third trimester and postpartum period ($t = 3.942$, $p < 0.001$). No other differences in sample time collection by trimester were detected.

There was a significant effect of pregnancy phase on hair cortisol levels ($F(3,20) = 3.9$; $p = 0.003$; One Way RM ANOVA; Figure 1B). Post-hoc tests revealed that hair cortisol levels were higher in the third trimester relative to the first trimester ($t = 4.1$, $p < 0.001$; Holm-Sidak) as well as the postpartum sample ($t = 2.9$, $p = 0.004$; Holm-Sidak). No other differences were detected.

3.3 Correlation between cortisol levels in saliva and hair

Correlation analysis examined the relationship between salivary cortisol as measured by AUCg and cortisol levels of concurrently collected samples of hair. Hair was collected on average at 15, 26 and 35.5 weeks prenatally and 12 weeks postpartum. Table 3 shows correlations between AUCg and hair cortisol levels. Note that only when AUCg was based on days collected at both the beginning as well as end of the interval reflecting hair growth (second and third trimesters) was there a statistically significant correlation between the salivary cortisol AUCg and concurrent hair cortisol level (Table 3). Finally, the overall mean AUCg and hair cortisol levels computed across all four sampling periods were significantly correlated ($r = 0.45$, $p = 0.047$).

3.4 Correlation between cortisol in new and long retrospective hair samples

A subset of the participants had sufficiently long hair samples from which to retrospectively analyze cortisol throughout their pregnancy ($n = 14$). The first 3 cm of hair from the full growth retrospective sample significantly correlated with the recent 3cm new growth sample cut in the third trimester ($R = 0.85$, $p < 0.001$) as expected. However, hair cut between 3-6 cm in the longer retrospective sample did not correlate with the 3 cm new growth sample cut at the end of the second trimester ($R = 0.17$, $p = 0.33$) three months earlier nor did hair cut between 6-9 cm in the long retrospective sample correlate with sample cut at the end of the first trimester ($R = -0.2$, $p = 0.29$). Thus as hair grows the cortisol levels in more distal portions do not correlate with recent growth.

4. Discussion

The goal of this study was to investigate maternal hair cortisol as a measure of longer term prenatal cortisol release. In support of this, cortisol levels in hair correlated with

contemporaneously collected salivary cortisol. Both salivary and maternal hair cortisol levels also displayed the expected increase across pregnancy followed by a postnatal decline. These findings provide valuable empirical support for the use of hair cortisol as a proxy measure of long term HPA activity during pregnancy.

The current study also followed hair and salivary cortisol levels *longitudinally* throughout pregnancy. Hair cortisol and salivary cortisol summarized as the AUCg were correlated during pregnancy, however, only in the 2nd and 3rd trimesters when saliva sampling spanned the period reflected by the hair cortisol were collected to compute the AUCg (six days of saliva sampling collected at the beginning and end of each trimesters). When saliva sampling was focused to a briefer period during the first trimester and postpartum period, hair cortisol did not correlate significantly with the AUCg. Salivary cortisol sampling *across* the trimester may be a better indication of cortisol reflected in hair cortisol than a single three day period not spanning the overall three month period.

Salivary cortisol is a response to acute conditions [30], more frequent collection of saliva over the day as well as across days provides a broader representation of cortisol activity over time. Furthermore as the first trimester and postpartum levels were collected over a single one week period, it is not surprising that correlations between hair and salivary cortisol were nonexistent. Restricting sampling to a one week period is much like a snap shot of the system whereas collecting early and late in the trimester increased reliability of that sampling [31] to reflect the full contribution of HPA activity to what is measured in hair. Much like a single cortisol sample which can be driven by situational factors, AUCg was derived from three days on which three samples were collected each day and are more reflective of individual patterns overall [32]. In addition, the overall mean of AUCg and hair cortisol levels computed across all four sampling periods was significantly correlated. Thus, conflicting relationships between cortisol output and chronic stress may be due to less frequent cortisol sampling [33]. Correlations between hair cortisol levels and salivary cortisol levels from the beginning and end of the trimester reflect HPA activity over the trimester in which cortisol levels are rising overall in association with pregnancy is ideal.

In the current study, participant adherence with saliva collection protocol was based on self-report of time of collection entered on the collection booklet. Self-report measures of adherence differ significantly from objective measures such as electronic monitoring by as much as 71-74% and thereby affect cortisol profiles [34, 35]. However others report that adherence as low as 81% does not effect on the diurnal cortisol profile [36]. Thus, while using electronic devices when collecting salivary cortisol is optimal, useful cortisol data can still be obtained in their absence. Consequently, use of cortisol determined from hair as a proxy measure of longer term cortisol levels avoids many confounding issues when collecting saliva, including timing of samples (afternoon vs. morning), adherence (collecting sample at appropriate times) and contamination (eating or drinking before taking sample, blood, etc.).

Hair samples collected during pregnancy provide access to HPA activity in the first trimester, a time previously hard to obtain because women may not realize they are pregnant or delay medical care until late in the first or early in the second trimester when they might be recruited for a study. Consequently the current study was able to only obtain three days of saliva samples in later part of the first trimester. In contrast hair was cut late in the first trimester and provided information regarding the activity of the HPA during the previous three months which has been previously unavailable.

In the current study, cortisol levels in saliva and hair rose throughout pregnancy, as previously demonstrated by saliva and serum [37-39]. Prenatal hair cortisol levels exhibited

a previously verified pattern unique to pregnancy suggesting that hair cortisol is a compelling reflection of cortisol release over a three month period. The elevated third trimester replicated cortisol levels found in hair [13] and in serum and saliva [37, 39, 40]. Hair cortisol as well as salivary cortisol also demonstrated a decline in the postpartum period, as previously documented [39]. The correlation of hair and salivary cortisol levels suggest hair cortisol levels are a useful marker of overall maternal HPA activity during each trimester. Hair provides a unique non-invasive means for assessing cortisol as a biomarker of long-term HPA-axis activity.

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References

- [1]. de Weerth C, Buitelaar JK. Physiological stress reactivity in human pregnancy--a review. *Neurosci Biobehav Rev.* 2005; 29:295–312. [PubMed: 15811500]
- [2]. Diego MA, Jones NA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, Gonzalez-Garcia A. Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosom Med.* 2006; 68:747–53. [PubMed: 17012528]
- [3]. Van den Bergh BRH, Van Calster B, Smits T, Van Huffel S, Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: A prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology.* 2007; 33:536–45. [PubMed: 17507916]
- [4]. Field T, Diego M. Cortisol: The culprit prenatal stress variable. *Int J Neurosci.* 2008; 118:1181–205. [PubMed: 18589921]
- [5]. Nepomnaschy PA, Welch KB, McConnell DS, Low BS, Strassmann BI, England BG. Cortisol levels and very early pregnancy loss in humans. *Proc Natl Acad Sci U S A.* 2006; 103:3938–42. [PubMed: 16495411]
- [6]. Gunnar MR. Quality of early care and buffering of neuroendocrine stress reactions: potential effects on the developing human brain. *Prev Med.* 1998; 27:208–11. [PubMed: 9578997]
- [7]. Coe CL, Lubach GR. Critical periods of special health relevance for psychoneuroimmunology. *Brain Behav Immun.* 2003; 17:3–12. [PubMed: 12615044]
- [8]. Gutteling BM, Weerth Cd, Buitelaar JK. Prenatal stress and children's cortisol reaction to the first day of school. *Psychoneuroendocrinology.* 2005; 30:541–9. [PubMed: 15808923]
- [9]. Ehlert U, Patalla U, Kirschbaum C, Piedmont E, Hellhammer DH. Postpartum blues: Salivary cortisol and psychological factors. *J Psychosom Res.* 1990; 34:319–25. [PubMed: 2342000]
- [10]. Nierop A, Bratsikas A, Klinkenberg A, Nater UM, Zimmermann R, Ehlert U. Prolonged salivary cortisol recovery in second-trimester pregnant women and attenuated salivary alpha-amylase responses to psychosocial stress in human pregnancy. *J Clin Endocrinol Metab.* 2006; 91:1329–35. [PubMed: 16434458]
- [11]. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 2000; 886:172–89. [PubMed: 11119695]
- [12]. Raul J-S, Cirimele V, Ludes B, Kintz P. Detection of physiological concentrations of cortisol and cortisone in human hair. *Clin Biochem.* 2004; 37:1105–11. [PubMed: 15589817]
- [13]. Kirschbaum C, Tietze A, Skoluda N, Dettenborn L. Hair as a retrospective calendar of cortisol production--Increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology.* 2009; 34:32–7. [PubMed: 18947933]

- [14]. Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH, Paus R. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal (HPA) axis and synthesize cortisol. *FASEB J*. 2005; 19:1332–4. [PubMed: 15946990]
- [15]. Cone EJ. Mechanisms of drug incorporation into hair. *Ther Drug Monit*. 1996; 18:438–43. [PubMed: 8857565]
- [16]. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. 2006; 147:255–61. [PubMed: 16483573]
- [17]. Yamada J, Stevens S, de Silva N, Gibbins S, Beyene J, Taddio A, Newman C, Koren G. Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. *Neonatology*. 2007; 92:42–9. [PubMed: 17356304]
- [18]. Kalra S, Einarson A, Karaskov T, Van Uum S, Koren G. The relationship between stress and hair cortisol in healthy pregnant women. *Clinical and Investigative Medicine*. 2007; 30:E103–7. [PubMed: 17716540]
- [19]. Kivlighan KT, DiPietro JA, Costigan KA, Laudenslager ML. Diurnal rhythm of cortisol during late pregnancy: Associations with maternal psychological well-being and fetal growth. *Psychoneuroendocrinology*. 2008; 33:1225–35. [PubMed: 18692319]
- [20]. Neu M, Goldstein M, Gao D, Laudenslager ML. Salivary cortisol in preterm infants: Validation of a simple method for collecting saliva for cortisol determination. *Early Hum Dev*. 2007; 83:47–54. [PubMed: 16766144]
- [21]. Kivlighan K, DiPietro JA, Costigan KA, Laudenslager ML. Diurnal rhythm of cortisol during late pregnancy: Associations with maternal psychological well-being and fetal growth. *Psychoneuroendocrinology*. 2008; 33:1225–35. [PubMed: 18692319]
- [22]. Schulze E, Laudenslager M, Coussons-Read M. An exploration of the relationship between depressive symptoms and cortisol rhythms in Colorado ranchers. *The Journal of Rural Health*. 2009; 25:109–13. [PubMed: 19166570]
- [23]. Beresford T, Arciniegas D, Alfors J, Clapp L, Martin B, Beresford H, Du Y, Liu D, Shen D, Davatzikos C, Laudenslager ML. Hypercortisolism in alcohol dependence and its relation to hippocampal volume loss. *Journal of Studies on Alcohol and Drugs*. 2006; 67:861–7.
- [24]. Riggs P, Thompson L, Tapert S, Fascella J, Mikulich-Culbertson S, Dalwani M, Laudenslager ML. Advances in Neurobiological Research Related to Interventions in Adolescents with Substance Use Disorders: Research to Practice. *Drug Alcohol Depend*. 2007; 91:306–11. [PubMed: 18038460]
- [25]. Society of Hair T. Statement of the Society of Hair Testing concerning the examination of drugs in human hair. *Forensic Sci Int*. 1997; 84:3–6.
- [26]. Wennig R. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int*. 2000; 107:5–12. [PubMed: 10689559]
- [27]. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003; 28:916–31. [PubMed: 12892658]
- [28]. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. 2006; 147:255–61. [PubMed: 16483573]
- [29]. Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Unm S. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clinical and Investigative Medicine*. 2007; 30:E183–E91. [PubMed: 17892760]
- [30]. Kirschbaum C, Hellhammer D. Salivary cortisol in psychoneuro-endocrine research: Recent developments and applications. *Psychoneuroendocrinology*. 1994; 19:313–33. [PubMed: 8047637]
- [31]. Kraemer HC, Giese-Davis J, Yutsis M, O'Hara R, Neri E, Gallagher-Thompson D, Taylor CB, Spiegel D. Design decisions to optimize reliability of daytime cortisol slopes in an older population. *American Journal of Geriatric Psych*. 2006; 14:325–33.

- [32]. Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State- and trait components. *Psychoneuroendocrinology*. 2007; 32:80–6. [PubMed: 17127010]
- [33]. Miller G, Chen E, Zhou E. It it goes up, must it come down? Chronic stress and hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull*. 2007; 133:25–45. [PubMed: 17201569]
- [34]. Kudielka BM, Broderick JE, Kirschbaum C. Compliance with saliva sampling protocols: Electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosom Med*. 2003; 65:313–9. [PubMed: 12652000]
- [35]. Broderick JE, Arnold D, Kudielka BM, Kirschbaum C. Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology*. 2004; 29:636–50. [PubMed: 15041086]
- [36]. Jacobs N, Nicolson NA, Derom C, Delespaul P, van Os J, Myin-Germeys I. Electronic monitoring of salivary cortisol sampling compliance in daily life. *Life Sci*. 2005; 76:2431–43. [PubMed: 15763075]
- [37]. Obel C, Hedegaard M, Henriksen TB, Secher NJ, Olsen J, Levine S. Stress and salivary cortisol during pregnancy. *Psychoneuroendocrinology*. 2005; 30:647–56. [PubMed: 15854781]
- [38]. Cohen M, Stiefel M, Reddy W, Laidlaw J. The secretion and disposition of cortisol during pregnancy. *J Clin Endocrinol Metab*. 1958; 18:1076–92. [PubMed: 13587623]
- [39]. Fleming AS, Ruble D, Krieger H, Wong PY. Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. *Horm Behav*. 1997; 31:145–58. [PubMed: 9154435]
- [40]. Carr B, Parker C, Madden J, MacDonald P, Porter J. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. *Am J Obstet Gynecol*. 1981; 139:416–22. [PubMed: 6258436]

Figure 1.

Hair and salivary AUCg cortisol increase during pregnancy. A) RM One Way ANOVAs revealed that first trimester salivary AUCg cortisol levels were lower in the first trimester relative to the second ($p=0.02$) and third trimesters ($p<0.001$). In addition, the postpartum period AUCg levels were lower than the first ($p=0.02$), second ($p<0.001$) and third trimesters ($p<0.001$). No other differences were detected. B) Third trimester hair cortisol levels are significantly elevated relative to first trimester (<0.001 ; One Way RM ANOVA) and postpartum levels (0.004 ; One Way RM ANOVA). No other differences were detected. Bars represent means $\pm$ SEM. * $p<0.05$, *** $p<0.001$

Figure 2.

Salivary and hair cortisol levels during pregnancy (ug/dl). A) Mean salivary cortisol levels at each collection time point by trimester during the perinatal period reflecting the expected diurnal curve across the day. Two Way RM ANOVA demonstrated a significant trimester x time interaction ($p=0.007$), such that there were differences in the wake+30 min collection time point between first and second trimester ($p<0.001$), first and third trimester ($p=0.001$), second trimester and postpartum period ($p<0.001$) and the third trimester and postpartum period ($p<0.001$). In addition, salivary cortisol levels at wake+30 min significantly differed from both lunch and wake+10 hour time points at every trimester. No other differences were detected. Error bars represent $\pm$ SEM. B) Individual salivary cortisol levels at waking +30 min sample across pregnancy and the postpartum period. The line connects the respective means of salivary cortisol at each stage of pregnancy. This figure indicates the expected increase during pregnancy as well as the variability across mothers during pregnancy that we have noted elsewhere [22].

Table 1

Maternal characteristics

Characteristics	Maternal	
	N	%
Age		
25-29	8	38.1
30-39	12	57.1
40-45	1	4.8
Race/Ethnicity		
Caucasian non-Hispanic/Latino	18	85.7
Hispanic/Latino	2	9.5
African-American	1	4.8
Employment status		
Unemployed	1	4.8
Part-time	7	33.3
Full-time	13	61.9
Years of education		
<12	0	0
12	1	4.8
>12	20	95.2
Marital status		
Living together, not married	3	14.3
Married	1	85.7

Table 2

Mean time of salivary sample collection each trimester. Times are calculated in minutes relative to self-report of waking time (mean $\pm$ standard deviation). There were no significant differences in saliva collection times across phases of pregnancy.

Time point	Wake+30 min	Lunch	Wake+10hr
1st Trimester	33.5 $\pm$ 6.3	330.1 $\pm$ 63.5	653.8 $\pm$ 80.2
2nd Trimester	33.2 $\pm$ 5.4	346.1 $\pm$ 45.8	659.2 $\pm$ 41.9
3rd Trimester	33.7 $\pm$ 5.0	337.4 $\pm$ 68.3	651.0 $\pm$ 80.4
Postpartum	32.6 $\pm$ 4.4	314.2 $\pm$ 65.4	637.1 $\pm$ 71.4

Table 3

Pearson correlation coefficients between maternal salivary cortisol AUCg and hair cortisol during the first, second and third trimesters of pregnancy and the postpartum period. Salivary cortisol AUCg and hair cortisol levels are correlated during the second and third trimesters.

Time point	Number of salivary sampling days	R ²	p value
1st Trimester	3	0.29	p=0.22
2 nd Trimester	6	0.43	p=0.04
3 rd Trimester	6	0.57	p=0.01
Postpartum- 12 wks	3	0.24	p=0.33