NIH Public Access
Author Manuscript
Am J Phys Anthropol. Author manuscript; available in PMC 2014 April 30.

Published in final edited form as:
Am J Phys Anthropol. 2012 October ; 149(2): 231–241. doi:10.1002/ajpa.22114.

Salivary Concentration of Progesterone and Cortisol Significantly Differs Across Individuals After Correcting for Blood Hormone Values

Shoko Konishi[1,2,*], **Eleanor Brindle**[3], **Amanda Guyton**[3], and **Kathleen A. O'Connor**[2,3]

[1]Department of Human Ecology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan

[2]Department of Anthropology, University of Washington, Seattle, WA 98195

[3]Center for Studies in Demography and Ecology, University of Washington, Seattle, WA 98195

Abstract

Between-individual variation of salivary progesterone (P4) and cortisol levels does not always closely reflect blood hormone concentrations. This may be partly a function of individual differences in salivary hormone excretion. We tested whether time of day at sampling and ethnicity contributed to individual variation in salivary hormones after adjusting for blood hormone levels. Forty-three Caucasian and 15 Japanese women (18–34 years) collected four sets of matched dried blood spot (DBS) and saliva specimens across a menstrual cycle (N = 232 specimen sets). Linear fixed-effects (LFE) models were used to estimate the effects of diurnal variation and ethnicity on salivary P4 and cortisol while adjusting for DBS levels. For each hormone, women with exclusively positive or negative residuals (unexplained variance) from the LFE models were categorized as high- or low-saliva-to-DBS hormone ratio (SDR; high or low salivary secretors), respectively. We found that salivary P4 ($P < 0.05$) was significantly higher in early morning compared to the afternoon, after controlling for DBS levels, ethnicity, and BMI. After further adjusting for this diurnal effect, significant individual variation in salivary P4 and cortisol remained: sixteen and nine women, respectively were categorized as low or high salivary secretors for both hormones ($P < 0.001$), suggesting systematic individual-specific variation of salivary hormonal concentration. We conclude that when saliva is used to quantify P4 or cortisol levels, time of day at sampling should be controlled. Even with this adjustment, salivary P4 and cortisol do not closely mirror between-individual variation of serum P4 and cortisol in a substantial proportion of individuals.

Keywords

biomarker; steroid hormone; dried blood spot

© 2012 WILEY PERIODICALS, INC.

[*]Correspondence to: Department of Human Ecology, Graduate School of Medicine, University of Tokyo, Shoko Konishi, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. moe@humeco.m.u-tokyo.ac.jp.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The low invasiveness of saliva collection together with the correlation with serum hormone levels has led to wide and growing use of saliva specimens for measuring steroid hormones including P4 and cortisol (Wood, 2009) in anthropological (Bentley et al., 1998; Vitzthum et al., 2002; Jasienska et al., 2006; Nunez-de la Mora et al., 2007) and biomedical studies (Vuorento et al., 1989; Yaneva et al., 2004; Chapman et al., 2009; Lachelin et al., 2009). Salivary P4 is a biomarker of ovarian function widely used in anthropological studies (Bentley et al., 1998; Vitzthum et al., 2002; Jasienska and Ellison, 2004; Nunez-de la Mora et al., 2007). Between-population variation of salivary P4 is thought to reflect variation in ovarian function which in turn is believed to represent variable environmental conditions in childhood and adulthood (Nunez-de la Mora et al., 2007; Vitzthum, 2008, 2009). Similarly, salivary cortisol is a biomarker of stress and metabolic function, secreted in a well-described diurnal pattern, widely used in biomedical, anthropological, and psychological studies (Flinn and England, 1997; Pruessner et al., 1999; Young and Breslau, 2004; Levine et al., 2007; Adam and Kumari, 2009).

This body of research on salivary P4 and cortisol is predicated on the assumption that salivary hormone levels closely reflect the total or the free hormone fraction circulating in the serum. Several studies have shown that salivary P4 is significantly correlated with free (Wang and Knyba, 1985) and total (Wang and Knyba, 1985; De Boever et al., 1986; Petsos et al., 1986; Vuorento et al., 1989; Wong et al., 1990; Kesner et al., 1992; Delfs et al., 1994) serum P4. In these studies single or multiple sets of matched serum and saliva specimens were collected from women across a menstrual cycle to calculate correlation coefficients between salivary and serum P4 concentrations (r range = 0.53–0.93). However, Thornburg et al. (2008) reported a correlation coefficient between individual salivary and serum P4 of only 0.17 in US women (N = 15) and 0.45 in Bolivians (N = 25) using Chatterton et al.' (2006) data. Like salivary P4, salivary cortisol shows significant correlation with free (Umeda et al., 1981; Vining et al., 1983) and total (Riad-Fahmy et al., 1982; Vining et al., 1983) serum cortisol levels (r range 0.89–0.97); the diagnostic value of salivary cortisol for Cushing's syndrome has been well established (Wood, 2009).

Despite these significant correlations, several studies indicate that between-individual variation in salivary hormone levels does not always closely reflect blood hormone concentrations. Petsos et al. (1986) collected 82 paired saliva and serum specimens from six women to compare salivary and serum P4 levels across women; regression analysis revealed different intercepts and slopes across individuals. A similar finding has been reported for cortisol; while some individuals showed high correlation between salivary cortisol and plasma free cortisol (r = 0.97), other individuals showed a much lower correlation (r = 0.49; Lewis, 2006). Moreover, McCracken and Poland (1989) reported that a saliva to serum cortisol ratio ranged widely across individuals, i.e., 0.01–0.03. Similarly, Delfs et al. (1994) reported that a saliva to plasma P4 ratio varied across individuals, i.e., 0.005–0.042. Thornburg et al. (2008) reported that the saliva to serum P4 ratio for each woman ranged from 0.62 to 70.7% in the US women and 0.37 to 8.4% in the Bolivian women. These studies suggest there is between-individual variation in the saliva to blood hormone ratio, and that between-individual variation in salivary hormone levels may reflect the saliva to blood hormone ratio as well as circulating blood hormone levels.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

It is usually assumed that between-population variation of salivary P4 reflects that of serum P4 levels, in other words, the saliva to serum hormone ratio is about the same across populations (Vitzthum, 2008, 2009). However, one study suggested that the saliva to serum P4 ratio may vary between populations; the saliva to serum P4 ratio was higher among US women (~0.03) compared to Bolivian women (~0.008), (Chatterton et al., 2006). As a result, salivary P4 of American women was twice as high as that of Bolivian women, whereas serum P4 was twice as high in Bolivian than in American women (Chatterton et al., 2006). The concentrations of corticosteroid-binding globulin (CBG) were not different between these populations, suggesting that the proportion of free serum P4 diffusing into saliva did not vary. The data suggest instead that P4 transfer from blood to saliva is higher in Caucasian than in Bolivian women, or inhibited in Bolivian women. In contrast, the saliva to serum cortisol ratio was similar between the two populations (0.033 for Bolivian and 0.029 for American women) suggesting that the lower apparent transfer into saliva is peculiar to P4 (Chatterton et al., 2006). As far as we know, there has been no further work exploring within or between population variation in saliva to blood hormone ratios, or potential contributing factors. Therefore, we were motivated to explore individual and population level variation in salivary and blood (specifically DBS) and in particular we focused on how it might be related to diurnal variation, ethnicity and anthropometric measures.

The factors that might be contributing to between-individual and between-population variation in the saliva to blood hormone ratio are not clear, but there are several correlates that may play contributing roles. Previous research suggests ethnicity, and time of day at specimen collection may affect the saliva to blood hormone ratio. One study suggested that the saliva to serum P4 ratio was higher among US women compared to Bolivian women (Chatterton et al., 2006). One study in which eight men collected hourly blood and saliva specimens from 8 am to 11 pm for 2 days found that the first morning saliva specimens contained disproportionately higher cortisol concentrations relative to serum (McCracken and Poland, 1989), suggesting that the saliva to blood cortisol ratio can be higher in the morning and lower in the afternoon. In contrast to the established diurnal variation of cortisol (e.g., Darne et al., 1989; McCracken and Poland, 1989; Allolio et al., 1990; Groschl et al., 2003; Liening et al., 2010), studies on diurnal variation of P4 have been inconclusive. Two studies reported higher salivary P4 concentration in the morning compared to the afternoon among children (Groschl et al., 2003) and adult men and women (Liening et al., 2010). It is not known whether salivary and blood P4 show diurnal variation among non-pregnant premenopausal women, or whether the saliva to blood hormone ratio changes during day. To further explore previous work finding that salivary measures of steroid hormones may not closely reflect blood concentrations, we examined paired salivary and blood P4 and cortisol measures collected across the day in a sample of Caucasian and Japanese women. We examined the effect of ethnicity, and time of day at sampling on the relationship of blood and saliva steroid hormone concentrations. We also explored anthropometric measures of body mass, as this has been found to be associated with cross-population differences in hormone levels (Randolph et al., 2003). We used a previously collected data set of paired saliva and dried blood spots across the menstrual cycle from US Caucasian women and women of Japanese ancestry residing in the US to explore

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

crosspopulation and crossindividual differences in saliva and dried blood spot measures of P4 and cortisol.

We tested three hypotheses: 1) salivary and DBS cortisol and P4 values would show similar diurnal patterns; with higher levels of P4 and cortisol expected in the morning, compared to those collected later during day, 2) whether Caucasian women would show higher salivary hormone concentrations compared to Japanese women, while controlling for differences in blood hormone concentrations, and 3) whether between-individual variation in salivary hormone concentrations would remain even after controlling for diurnal variation, body mass, ethnicity, and blood hormone concentration.

We found that salivary excretion of P4 and cortisol appears diurnal, over and above the diurnal pattern of cortisol in blood. After further controlling for this additional diurnal variation (and ethnicity and BMI), significant individual variation in P4 and saliva remained, consistent with an intriguing pattern we refer to as a high vs. low “secretors.”

MATERIALS AND METHODS

Study participants and specimen collection

Study participants were recruited in Seattle, Washington, using flyers, and advertisements in local websites, newspapers, and magazines. Eligible women were aged 18–34 years, had not been on hormonal contraceptives or hormonal medications within the past 3 months, and were not currently pregnant or breastfeeding. No eligibility criteria based on cycle regularity or length, or BMI, were used. To examine population differences in salivary excretion of P4 and cortisol, participants were limited to either Caucasian or Japanese ethnicity; ethnicity was self-identified by the participants.

Participants gave written informed consent upon enrollment and filled out a questionnaire on demographic characteristics at the Biodemography Laboratory, University of Washington. Height was measured to the nearest 0.1 cm using a Seca 214 portable stadiometer (Seca, Hamburg, Germany). Body weight and percent body fat to the nearest 0.1 kg and 0.1%, respectively were measured using an Inner Scan Body Composition Monitor (Tanita, Tokyo, Japan). Skinfold thicknesses at biceps and triceps were measured twice for each site with a Lange skinfold caliper (Beta Technology, Santa Cruz, CA) with a precision of 2 mm. Mean of the two skinfold measurements for each site were used for analyses. Circumferences at waist (WC) at the narrowest point, hip (HC) at the widest point and mid-upper arm (MUAC) were measured with a plastic tape, with a precision of 1 mm. BMI was calculated as [weight (kg)]/[height (m)]^2.

Matched blood and saliva specimens were collected by two sampling strategies: once-a-week (N = 50 women enrolled) and every-day sampling (N = 11 women enrolled). Forty-eight women completed matched blood and saliva specimen collection once a week for four consecutive weeks at the Biodemography Laboratory, University of Washington. Ten women completed matched specimen collection every day at their home for 40 consecutive days starting on the first or second day of menstruation. Mean (SD) of cycle length was 29.8 (5.2) days (range 16–46) for 41 cycles for which starting date of two consecutive cycles

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

could be obtained during the study. It was 32.1 (16.5) days (range 17–75) for right-censored cycles (N = 17) for which starting date of only one cycle could be obtained.

Finger prick blood (DBS) specimens were collected on filter paper (Whatman 903 Protein Saver Card), dried at room temperature for 4–18 h, and stored in a home freezer (at home) or at −20°C (in the lab) until assay. After the mouth was rinsed with water, saliva specimens of ~2 mL were collected via passive drool into polypropylene vials and stored in a home freezer (at home) or at −20°C (in the lab) until assay. For once-a-week sampling, time of day at sampling, time of last food or drink (except for water and other noncaloric drink) and the most recent date of starting menstruation was recorded at collection. For every-day sampling, time of day at sampling and presence or absence of menstrual bleeding was recorded at collection. All specimens were collected when convenient for the participants, which ranged between = am and 6 pm. Sampling started in May 2010 and ended in August 2010. After completing specimen collection at home, the every-day sampling participants brought their DBS and saliva specimens to the lab in a cooler with frozen icepacks. All the specimens were stored at −20°C in the lab until analysis. All procedures were approved by the Institutional Review Board of the University of Washington.

Laboratory analyses

Prior to the present study, a validation study was conducted for DBS P4 and cortisol, using the every-day sampling specimens in addition to other sets of matched specimens collected at the Biodemography Laboratory, University of Washington, for validation purposes. Using the matched serum and DBS specimens, it was confirmed that DBS P4 was highly positively associated with serum P4 ([DBS P4 (ng mL^{-1})] = 1.032 × [serum P4 (ng mL^{-1})] + 2.120, R^2 = 0.982; Fig. 1A; N = 9 pairs; unpublished data) and that DBS cortisol was highly positively associated with serum cortisol ([DBS cortisol (ng mL^{-1})] = 1.109 × [serum cortisol (ng mL^{-1})] − 4.026, R^2 = 0.833; Fig. 1B; N = 51 pairs; unpublished data). Figure 2 illustrates mean DBS P4 concentration across one menstrual cycle from nine women who participated in the every-day sampling, aligned by an estimated day of ovulation; one participant who did not ovulate during the sampling period was excluded. Presence or absence of ovulation and day of ovulation was estimated by Kassam method (O'Connor et al., 2006) using pregnanediol glucuronide (the major urinary metabolite of P4) concentration in urine specimens collected simultaneously with the DBS and saliva specimens.

For the present article, four specimen sets (on cycle days 3, 10, 17, and 24) from each of every-day sampling participant were analyzed so that every participant (N = 58) contributed four specimen sets (N = 232). In the lab, saliva specimens were thawed and centrifuged at 2800 RPM for 20 min, and the aqueous layer was removed and stored at −20°C until assay. The DBS specimens were assayed for P4 using an in-house enzyme immunoassay (EIA) using monoclonal antibody CL-425 (Graham et al., 2001). The assay crossreacts 100, 188, 172, 147, 94, 64, 55, and 12.5% with P4, 4-pregnen-3α-ol-20-one, 4-pregnen-3β-ol-20-one, 4-pregnen-11α-ol-3,20-dione, 5α-pregnan-3β-ol-20-one, 5α-pregnan-3α-ol-20-one, 5α-pregnan-3,20-dione, and 5β-pregnan-3β-ol-20-one, respectively, and crossreacts <10% with other steroids tested (Graham et al., 2001). The lower limit of detection of the in-house P4 EIA is 0.1 ng mL^{-1}. All DBS specimens were run at a 1:50 dilution; two 1/8″ inch punches

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

of DBS were eluted with 152.5 μL of 0.1% BSA blocking buffer and incubated overnight at 4°C. On the next day, after 30 min of shaking, the eluent was assayed for P4. All of a woman's specimens were placed on the same plate except for assay reruns. For DBS P4 in-house controls, between assay CVs were 16.7 and 11.6% and within assay CVs were 5.4 and 6.5%, respectively for the low (0.33 ng mL^{-1}) and high (1.43 ng mL^{-1}) controls (N = 43 plates).

The salivary specimens were assayed for P4 using Salimetrics Progesterone Salivary Assay Kits (Salimetrics LLC, PA). The lower limit of detection is 0.01 ng mL^{-1}. All of a woman's specimens were placed on the same plate except for assay reruns. All saliva specimens were run undiluted. For salivary P4 kit controls, between assay CVs were 5.9 and 6.8% and within assay CVs were 4.8 and 6.5%, respectively for the low (24.1 pg mL^{-1}) and high (727.4 pg mL^{-1}) controls (N = 7 plates).

The DBS and saliva specimens were assayed for cortisol using an in-house EIA using polyclonal antibody R4866 (Munro and Stabenfeldt, 1985; Trumble et al., 2010; O'Connor et al., 2011). The assay crossreacts 100% with cortisol and <10% with other steroids tested. The lower limit of detection is 0.078 ng mL^{-1}. All DBS specimens were run at a 1:40 dilution; three 1/8″ inch punches of DBS were eluted with 183 μL of 0.1% BSA blocking buffer and incubated overnight at 4°C. On the next day, after 30 min of shaking, the eluent was assayed for cortisol. Saliva specimens were run either at 1:2 or 1:10 dilutions in 0.1% BSA blocking buffer. For cortisol assays, matched DBS and saliva specimens were assayed on the same plate except for assay reruns. All of a woman's specimens were placed on the same plate except for assay reruns. For DBS cortisol in-house controls, between assay CVs were 13.0 and 5.9% and within assay CVs were 6.7 and 3.9%, respectively for the low (0.47 ng mL^{-1}) and high (2.59 ng mL^{-1}) controls (N = 25 plates). For salivary cortisol in-house controls, between assay CVs were 11.6 and 8.5% and within assay CVs were 4.3 and 3.9%, respectively for the low (0.36 ng mL^{-1}) and high (1.50 ng mL^{-1}) controls (N = 24 plates).

All the DBS and saliva specimens fell within the limit of detection for the P4 and cortisol assays except for one DBS specimen, which was under the limit of detection (~0.078 ng mL^{-1}) for cortisol; this set of matched DBS and saliva was excluded from further statistical analyses.

Statistical analyses

Age and anthropometric measures were compared between Caucasian vs. Japanese women using t tests. Distribution of the time of day at sampling was compared between the two ethnic groups using Fisher's exact test. The concentrations of each hormone (P4 or cortisol) in each specimen type (DBS or saliva) were compared between Caucasian vs. Japanese women using Wilcoxon's rank sum test, because distributions of hormone concentrations were skewed.

Time of day at sampling was categorized into three intervals: early morning (5–9 am), late morning (9 am–noon), and afternoon (noon–6 pm). For each hormone (P4 or cortisol) in each specimen type (DBS or saliva), concentrations were compared between early vs. late morning, and early morning vs. afternoon, using Wilcoxon's rank sum test.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Pearson correlation coefficients were calculated to examine the correlation between salivary and DBS hormone (separately for P4 and cortisol) levels and between P4 and cortisol concentrations (separately for DBS and saliva) using logged values.

The LFE model (multiple linear regression) analyses were conducted with either salivary P4 or salivary cortisol levels as the dependent variable, and corresponding DBS hormone values (P4 or cortisol, respectively), time of day at sampling (early morning, late morning, or afternoon), ethnicity (Japanese or Caucasian), and BMI as the independent variables. Because linear relation was expected between DBS and salivary hormone concentrations, unlogged hormone values were used for all the statistical analyses. The distribution of time of day at sampling did not differ between Japanese and Caucasian women (Fisher's exact test; $P = 0.369$). These LFE models do not take into account individual differences in baseline hormone levels, which might confound the association between salivary hormone concentration and the covariates. Therefore, the same sets of data were also analyzed using linear mixed-effects (LME) models with participants modeled as random effects. The LME models control for the nonindependence of repeated specimens from each participant (West et al., 2007; Trumble et al., 2010), thus eliminating between-individual variation of residuals.

To examine individual differences in salivary hormone levels after adjusting for DBS hormone concentration, ethnicity, BMI, and time of day at sampling, residuals of the LFE model analyses were calculated (separately for P4 and cortisol). Because individual variation of residuals, calculated as difference between observed and expected salivary hormone levels, were the focus of the analysis, LME models were not used here. Using the residuals of either salivary P4 or cortisol LFE models, participants were categorized into high-, medium-, or low-saliva-to-DBS hormone ratio (SDR) of each salivary hormone. Participants whose residuals were positive or negative for all four specimen sets are either high- or low-SDR, respectively. Participants with both positive and negative residuals are called medium-SDR. For each hormone, using Fisher's exact test, the observed distribution of SDR categories was compared against the expected distribution of categories to test whether there was significant individual variation in residual distributions. The SDR status for salivary P4 and cortisol for each person was crosstabulated to test whether the high- (or low-) SDR for P4 were also high- (or low-) SDR for cortisol. Statistical significance of the contingency between SDR status for P4 and cortisol was determined using Fisher's exact test.

Pearson correlation coefficients were calculated for DBS and salivary hormone levels by SDR category (separately for P4 and cortisol) using logged values.

All statistical analyses were conducted using R 2.12.0 (R Development Core Team, 2010). The level of statistical significance was defined as $P < 0.05$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

RESULTS

Participant and specimen characteristics

Sixty-one women were enrolled in the study and 58 women completed sampling sessions, yielding a total of 232 sets of matched DBS and saliva specimens for analyses. Sixty percent ($N = 9$) of the Japanese participants were born in Japan, while 40% ($N = 6$) were born in the US; this sample will be referred to subsequently as Japanese ancestry women. Participant age ranged from 18 to 33 years, with a mean (SD) of 24.0 (4.4); and Caucasian women showed significantly higher BMI compared to Japanese women [23.1 (4.0) vs. 20.4 (2.0), $P = 0.001$] (Table 1). There were no significant differences in DBS or salivary hormone levels between Japanese and Caucasian women (Table 1). Out of the 232 specimen sets, 54, 92, and 86 sets were collected in the early morning, late morning, and afternoon, respectively (Table 1).

Correlation between DBS and salivary P4 and cortisol

The correlation between DBS and salivary P4 ($r = 0.345$; $P < 0.001$) was weaker than that of DBS and salivary cortisol ($r = 0.636$; $P < 0.001$) (Fig. 3). These correlations may be affected by the fact that DBS and salivary P4 were measured in different assays, while DBS and salivary cortisol were measured in the same assay. While there was a modest correlation between DBS P4 and cortisol ($r = 0.277$; $P < 0.001$), that of salivary P4 and cortisol showed a much stronger correlation ($r = 0.646$; $P < 0.001$) (Fig. 3).

Time of day at sampling and hormone levels

DBS P4 concentration did not differ by time of day at sampling, while salivary P4 levels collected in early morning were significantly higher than those from late morning ($P < 0.05$) and the afternoon ($P < 0.001$) (Table 2). Cortisol concentrations in both DBS and saliva specimens collected in the early morning were significantly higher than those collected in the late morning and afternoon ($P < 0.001$) (Table 2).

After adjusting for DBS hormone levels, ethnicity, and BMI in LFE models, saliva specimens collected in early morning continued to show higher P4 concentrations compared to those collected in the afternoon (Table 3). When LME models were used (controlling for correlation among specimens contributed by individuals), saliva specimens collected in early morning showed higher cortisol, but not higher P4 concentrations, compared to those collected in the afternoon (Table 3). We interpret this as suggesting that the observed diurnal variation of salivary P4 could partly be attributable to the different SDR categories, i.e., secretor categories, of individuals, while diurnal variation of salivary cortisol was evident even after adjusting for the between-individual variation of salivary cortisol.

Ethnicity was not significantly associated with salivary P4 or cortisol in either LFE or LME models (Table 3).

High-, medium-, and low-SDR for salivary P4 and cortisol

Using the residuals from the LFE models, described in Table 3, each participant was categorized into three SDR categories for each hormone (Table 4). Assuming random

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

variation of residuals across individuals, the expected number of high-SDR can be calculated as 58 persons * (0.5)^4 = 3.6 persons (= [number of participants] multiplied by [probability of having positive residuals for 4 specimen sets]).The expected number of low-SDR is also 3.6 persons, and thus that of medium SDR is 58 − (3.6*2) = 50.8 persons. The observed distribution of SDR categories significantly differed from the expected values ($P < 0.001$) for both hormones. Therefore, there were significant between-individual differences in salivary hormone concentrations even after DBS hormone concentrations, time of day at sampling, ethnicity, and BMI was adjusted.

Respectively, 9 and 16 participants were categorized as high- and low-SDR for both P4 and cortisol, whereas there was only one participant categorized as high-SDR for one hormone and low-SDR for the other hormone (Table 4). Moreover, there was significant contingency between the SDR categories for the two hormones ($P < 0.001$). In other words, women who showed higher P4 in saliva also showed higher cortisol in saliva, and vice versa, irrespective of DBS hormone levels.

Correlations between salivary and DBS hormone levels are shown by SDR category for each hormone (Fig. 4); the correlation between salivary and DBS hormone levels is higher within the subgroups (i.e., Fig. 3). Correlation coefficients between DBS and salivary P4 were 0.533, 0.652, and 0.695, respectively, for the low-, medium-, and high- P4 SDR group ($P < 0.001$ for all). Correlation coefficients between DBS and salivary cortisol were 0.510, 0.821, and 0.757, respectively, for the low-, medium-, and high- cortisol SDR group ($P < 0.001$ for all). For each hormone the salivary levels varied widely across the SDR groups, while the DBS hormone values overlapped across the SDR groups.

DISCUSSION

The SDR (secretor) categories for salivary P4 and cortisol showed significant consistency within each individual, which suggests that salivary concentrations of these steroid hormones depend on individual-specific characteristics perhaps salivary hormone excretion capacity, independent of DBS hormone levels. Further work is needed to clarify the factors that cause individual variations in saliva to blood hormone ratios. Possible factors may include steroid binding proteins, genetic factors, or secretory mechanics. Long-term (i.e., months or years) consistency of SDR categories should also be examined, and other steroid hormones such as estrogens and testosterone should be evaluated for their saliva to blood hormone ratios. Our work suggests that salivary P4 or cortisol may not be an ideal biomarker when between-individual or between-population differences in circulating blood P4 or cortisol levels are of interest. It also suggests that if salivary P4 or cortisol are used as indicators of within-individual variation in serum hormone levels, LME models should be used to control for any potential individual-specific differences in salivary P4 or cortisol. Time of day of collection (for blood and other diurnal effects) should be controlled for as well.

We did not find any individual variation in blood or salivary hormones relative to ethnicity in our sample, despite significant differences in the samples in body composition measures. An ethnic difference in the saliva to blood P4 ratio like that reported by Chatterton et al.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(2006) was also not found. This may be due to the fact that both Caucasian and Japanese women of our study were residing in the same city, whereas Chatterton et al.'s participants lived in the US and Bolivia. Nunez-de la Mora et al. (2007) suggested that the environmental conditions during childhood and adolescence may influence salivary P4 levels during adulthood. Such effects cannot be tested with our data due to the small sample size. Nine of the Japanese participants were born in Japan, while six were born in the US. Although the samples differed in body mass, fat and skinfold measurements, we found no effects of BMI or other anthropometric measures (body fat) on relationships between serum and salivary P4 or cortisol.

We found significant and complex effects of diurnal variation in salivary excretion of both P4 and cortisol. In our sample salivary P4 level was higher in early morning compared to late morning and afternoon specimens, although no such diurnal variation was observed in DBS P4 levels. This supports the existence of diurnal variation in salivary P4 but not in DBS P4, although the LME model showed no significant diurnal variation of salivary P4, suggesting that observed diurnal variation of salivary P4 was confounded by individual differences in P4 SDR (secretors) category. Both salivary and DBS cortisol levels showed diurnal variation, as expected; however, salivary cortisol levels were higher in early morning compared to afternoon specimens, even after adjusting for the diurnal change of DBS cortisol levels using an LME model. Thus, both the cortisol and P4 data suggest that other sources of diurnal variation, over and above that seen in the blood, may be at play, and confounding our understanding of relationships between salivary and DBS/blood hormone concentrations.

To the best of our knowledge, the present study is the first to show a diurnal rhythm in salivary P4 concentration among nonpregnant women using multiple specimens collected from each individual. One study targeting children aged 2–15 years showed a circadian rhythm of salivary P4 with the highest values in the morning, followed by noon and evening specimens (Groschl et al., 2003). On the other hand, one study targeting late pregnant women did not detect any diurnal variation in salivary P4 levels (Scott et al., 1990). Another study targeting late pregnant women showed that serum P4 concentrations were significantly lower in the morning (9 am) than in the evening (7 pm), but the diurnal rhythm diminished after delivery (Allolio et al., 1990). These results suggest that controlling for time of day of saliva collection is important in analyses using salivary P4. Future work should validate and examine this in additional samples.

Interestingly, individuals who had a high-SDR for P4 (high salivary P4 secretors) were also more likely to also have a high-SDR for cortisol (high salivary cortisol secretion), with the same pattern observed for low-SDR, even after controlling for diurnal variation. As a result, correlation between salivary P4 and cortisol ($r = 0.646$, $P < 0.001$) was much stronger than that between DBS P4 and cortisol ($r = 0.277$, $P < 0.001$). Wirth et al. (2007) did not find any significant correlation between salivary P4 and cortisol levels among women who were not on oral contraceptive pills, but they did find a significant correlation among men, and women who used oral contraceptives. Liening et al. (2010) found a positive correlation ($r = 0.30$, $P \leq 0.05$) between salivary P4 and cortisol among women (including oral contraceptive users), but in only one of the two data collecting sessions. However, these

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

studies did not measure P4 and cortisol levels in blood. Our results imply that part of the correlation between salivary P4 and cortisol hormones may be due to salivary excretion dynamics and other factors, independent of blood levels.

Our results indicate that the saliva to blood cortisol ratio decreases during the day. While diurnal variation of both blood and salivary cortisol has been well documented (e.g., Darne et al., 1989; McCracken and Poland, 1989; Allolio et al., 1990; Groschl et al., 2003; Liening et al., 2010), studies on the saliva to blood cortisol ratio with respect to diurnal variation have been scarce. McCracken and Poland (1989) reported that among eight men first morning saliva specimens contained disproportionately high cortisol concentrations relative to serum, which is consistent with our results. They discuss that cortisol might be sequestered in the salivary gland or in the oropharynx during the night, although the exact reasons remain to be determined (McCracken and Poland, 1989). The mechanisms contributing to variation across individuals in the saliva to blood hormone ratio cannot be clarified in our study, but our results suggest that we need to control time of day at saliva collection for cortisol analyses, as well as for P4 analyses.

Postprandial increases of cortisol in saliva (Gibson et al., 1999) and in serum (Slag et al., 1981) were judged not to affect our findings. While DBS and salivary cortisol were higher within 1 h after a meal, after controlling for diurnal variation, no such effect was seen in DBS or salivary P4 (analysis with once-a-week sampling data; results not shown). Although the postprandial effect may have weakened the observed correlation between salivary P4 and cortisol, we still found a highly significant correlation. Menstrual cycle variability was also judged not to affect our findings, because all women gave a set of matched specimens once a week for four consecutive weeks so that each woman gave specimens at both follicular and luteal phases of a menstrual cycle. Anovulatory cycles, if any, were judged irrelevant to our findings, because statistical analyses were conducted without taking cycle phase into account. Women with BMI <18.5 ($N = 4$) or >30 ($N = 2$) showed cycle length ranging from 26 to 36 days, while apparently anovulatory women ($N = 2$) judged from very long cycle lengths (>73 days) had normal BMI (20.3 and 21.1). Therefore, low or high BMI is judged not to be related with anovulation in the present samples. Temporal variation in the collection of saliva and DBS specimens cannot be a confounding factor in the present study because the once-a-week sampling participants ($N = 48$) collected saliva immediately before DBS. Although the every-day sampling participants ($N = 10$) were not instructed about the order of specimen collection, it is unlikely it confounded our findings. In addition, analyses only using the once-a-week sampling data did not significantly change the findings (results not shown).

Most of the previous studies that examined the correlation between blood and salivary P4 levels (Wang and Knyba, 1985; De Boever et al., 1986; Petsos et al., 1986; Vuorento et al., 1989; Wong et al., 1990; Kesner et al., 1992; Delfs et al., 1994) used radioimmunoassay (RIA) to quantify P4 in both saliva and blood specimens, except that Delfs et al. (1994) used EIA for saliva and RIA for serum and De Boever et al. (1986) used a chemiluminescence immunoassay (CLIA) for saliva and RIA for serum. Extraction of saliva (Wang and Knyba, 1985; Kesner et al., 1992), serum (De Boever et al., 1986), or both saliva and serum (Vuorento et al., 1989; Wong et al., 1990) were done prior to the assays, while the other

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

studies (Petsos et al., 1986; Delfs et al., 1994) including ours did not extract saliva or blood specimens. In these previous studies (Wang and Knyba, 1985; De Boever et al., 1986; Petsos et al., 1986; Vuorento et al., 1989; Wong et al., 1990; Kesner et al., 1992; Delfs et al., 1994) the correlation coefficients for salivary and serum P4 ranged from 0.53 (Wang and Knyba, 1985) to 0.93 (Wong et al., 1990), whereas the correlation was slightly weaker in our results ($r = 0.345$; $P < 0.001$). Possible reasons for the low correlation include the use of different assays for quantifying salivary and DBS P4 and use of nonextracted specimens. The matrix and viscosity of nonextracted saliva could vary across the day, potentially influencing the matrix in assays. However, when DBS and salivary P4 correlation was calculated for each SDR (secretor) category, *r* became much higher (range 0.533–0.695; Fig. 4), which suggests that the observed low correlation between DBS and salivary P4 levels can partly be attributed to individual differences in saliva to blood hormone ratio. Therefore, the relatively lower correlation between DBS and salivary P4 levels in our study does not weaken the importance of our findings.

While the cross-reactants of the monoclonal antibody CL-425 used for P4 EIA (Graham et al., 2001) might reduce overall correlations between salivary and DBS P4, they should not affect our main findings. Our results show that SDR categories tend to be same for both P4 and cortisol within each individual, which effectively excludes any confounding effects from the P4 crossreactants.

A limitation of our study is that we did not measure the free fraction of P4 or cortisol or CBG levels in DBS. Saliva hormone levels reflect the free fraction of steroid hormones in blood (Umeda et al., 1981; Wang and Knyba, 1985). However, several studies suggest that salivary P4 and cortisol levels are highly positively correlated with both free and total serum P4 and cortisol, respectively. Wang and Knyba (1985) measured free P4 levels using equilibrium dialysis, in which salivary P4 showed a significantly positive correlation between both free ($r = 0.75$, $P < 0.001$) and total ($r = 0.78$, $P < 0.001$) serum P4. Gozansky et al. (2005) found that salivary cortisol closely paralleled both free and total serum cortisol when serum total cortisol is below the average CBG saturation point (~180 ng mL^{-1}), (Brien, 1981). All the DBS specimens in our study showed cortisol levels below the CBG saturation point, which suggests that total and free blood P4 should be similar in terms of correlation with salivary P4. In addition, the equation for calculating unbound plasma cortisol concentration (Coolens et al., 1987) predicts that individual variation of CBG within a normal range for naturally cycling women (95% confidence intervals: ~33–47 μg mL^{-1}), (Coolens et al., 1987) contributes only up to a 40% difference in free serum cortisol concentrations, which is much smaller than the salivary cortisol differences observed between high- and low-SDR in our study. While median cortisol concentrations in DBS were 37.9 and 43.9 ng mL^{-1} for low- and high-SDR (secretors), respectively (16% difference), those in saliva were 4.0 and 13.5 ng mL^{-1}, respectively (238% difference). Therefore, we suspect that factors such as excretion rates into saliva, in addition to the free hormone levels in blood, affects P4 and cortisol concentrations in saliva. Although our current data do not provide support for effects from binding protein dynamics in circulation, this may merit further investigation.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

CONCLUSIONS

We found strong diurnal patterning in two salivary hormones, P4 and cortisol. Consequently, when saliva is used to quantify P4 or cortisol levels, time of day at sampling should be controlled. This diurnal pattern persists even after diurnal blood levels are controlled for, suggesting that some other factor(s) associated with saliva are contributing to diurnal salivary secretion. Further, we found that after controlling for blood hormone levels, the excess salivary diurnal variation, ethnicity, and BMI, significant patterned individual variation in salivary P4 and cortisol levels remained; some individuals consistently showed higher or lower levels of secretion of both salivary P4 and cortisol suggesting it is something inherent to the saliva. Taken together, these results support that though significantly correlated, salivary and blood hormone levels do not always closely mirror each other, due to diurnal effects of both hormones and saliva, and apparently other currently not understood processes. We found that salivary P4 and cortisol do not closely mirror between-individual variation of serum P4 and cortisol in a substantial proportion of individuals. Further work is needed to clarify factors that cause individual variations in salivary secretion of hormones, diurnal variation in salivary hormones, and variation in the saliva to blood hormone ratio.

Acknowledgments

The authors thank the participants for their contributions. They also thank J. Shofer, B. Trumble, T. Hayes Constant, and K. Konishi for their advice and support for this research.

Grant sponsor: KAKENHI; Grant number: 21790572. Grant sponsor: NICHD; Grant number: R24 HD42828.

Abbreviations

DBS	dried blood spot
P4	Progesterone
SDR	saliva-to-DBS hormone ratio
SE	standard error

LITERATURE CITED

Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinol. 2009; 34:1423–1436.

Allolio B, Hoffmann J, Linton EA, Winkelmann W, Kusche M, Schulte HM. Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing-hormone. Clin Endocrinol. 1990; 33:279–289.

Bentley GR, Harrigan AM, Ellison PT. Dietary composition and ovarian function among Lese horticulturalist women of the Ituri Forest, Democratic Republic of Congo. Eur J Clin Nutr. 1998; 52:261–270. [PubMed: 9578338]

Brien TG. Human corticosteroid binding globulin. Clin Endocrinol. 1981; 14:193–212.

Chapman JF, McIntyre MH, Lipson SF, Ellison PT. Weight change and ovarian steroid profiles in young women. Fertil Steril. 2009; 91:858–861. [PubMed: 18314121]

Chatterton RT, Mateo ET, Lu D, Ling FJH. Interpopulational differences in the concentrations and ratios of salivary and serum progesterone. Fertil Steril. 2006; 86:723–725. [PubMed: 16828474]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Coolens J, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. J Steroid Biochem. 1987; 26:197–202. [PubMed: 3560936]

Darne FJ, McGarrigle HHG, Lachelin GCL. Diurnal variation of plasma and saliva oestrogen, progesterone, cortisol and plasma dehydroepiandrosterone sulphate in late pregnancy. Eur J Obstet Gynecol Reprod Biol. 1989; 32:57–66. [PubMed: 2776957]

De Boever J, Kohen F, Vandekerckhove D. Direct solid-phase chemiluminescence immunoassay for salivary progesterone. Clin Chem. 1986; 32:763–767. [PubMed: 3516449]

Delfs TM, Naether OG, Klein S, Leidenberger FA, Fottrell P, Zimmermann RC. 24-hour profiles of salivary progesterone. Fertil Steril. 1994; 62:960–966. [PubMed: 7926142]

Flinn MV, England BG. Social economics of childhood glucocorticoid stress response and health. Am J Phys Anthropol. 1997; 102:33–53. [PubMed: 9034037]

Gibson EL, Checkley S, Papadopoulos A, Poon L, Daley S, Wardle J. Increased salivary cortisol reliably induced by a protein-rich midday meal. Psychosom Med. 1999; 61:214–224. [PubMed: 10204975]

Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. Clin Endocrinol. 2005; 63:336–341.

Graham L, Schwarzenberger F, Möstl E, Galama W, Savage A. A versatile enzyme immunoassay for the determination of progestogens in feces and serum. Zoo Biol. 2001; 20:227–236.

Groschl M, Rauh M, Dorr HG. Circadian rhythm of salivary cortisol, 17 alpha-hydroxyprogesterone, and progesterone in healthy children. Clin Chem. 2003; 49:1688–1691. [PubMed: 14500602]

Jasienska G, Ellison PT. Energetic factors and seasonal changes in ovarian function in women from rural Poland. Am J Hum Biol. 2004; 16:563–580. [PubMed: 15368604]

Jasienska G, Thune I, Ellison PT. Fatness at birth predicts adult susceptibility to ovarian suppression: an empirical test of the predictive adaptive response hypothesis. Proc Natl Acad Sci USA. 2006; 103:12759–12762. [PubMed: 16908839]

Kesner JS, Wright DM, Schrader SM, Chin NW, Krieg EF. Methods of monitoring menstrual function in field studies: efficacy of methods. Reprod Toxicol. 1992; 6:385–400. [PubMed: 1463919]

Lachelin GCL, McGarrigle HHG, Seed PT, Briley A, Shennan AH, Poston L. Low saliva progesterone concentrations are associated with spontaneous early preterm labour (before 34 weeks of gestation) in women at increased risk of preterm delivery. BJOG. 2009; 116:1515–1519. [PubMed: 19614937]

Levine A, Zagoory-Sharon O, Feldman R, Lewis JG, Weller A. Measuring cortisol in human psychobiological studies. Physiol Behav. 2007; 90:43–53. [PubMed: 17055006]

Lewis J. Steroid analysis in saliva: an overview. Clin. Biochem Rev. 2006; 27:139–146.

Liening SH, Stanton SJ, Saini EK, Schultheiss OC. Salivary testosterone, cortisol, and progesterone: two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels. Physiol Behav. 2010; 99:8–16. [PubMed: 19833145]

McCracken J, Poland R. Saliva and serum cortisol dynamics following intravenous dexamethasone in normal volunteers. Life Sci. 1989; 45:1781–1785. [PubMed: 2593767]

Munro C, Stabenfeldt G. Development of a cortisol enzyme immunoassay in plasma. Clin Chem. 1985; 31:956.

Nunez-de la Mora A, Chatterton RT, Choudhury OA, Napolitano DA, Bentley GR. Childhood conditions influence adult progesterone levels. PLoS Med. 2007; 4:813–821.

O'Connor KA, Brindle E, Miller RC, Shofer JB, Ferrell RJ, Klein NA, Soules MR, Holman DJ, Mansfield PK, Wood JW. Ovulation detection methods for urinary hormones: precision, daily and intermittent sampling and a combined hierarchical method. Hum Reprod. 2006; 21:1442–1452. [PubMed: 16439502]

O'Connor KA, Brindle E, Shofer J, Trumble BC, Aranda JD, Rice K, Tatar M. The effects of a long-term psychosocial stress on reproductive indicators in the baboon. Am J Phys Anthropol. 2011; 145:629–638. [PubMed: 21702002]

Petsos P, Ratcliffe WA, Heath DF, Anderson DC. Comparison of blood spot, salivary and serum progesterone assays in the normal menstrual cycle. Clin Endocrinol. 1986; 24:31–38.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Pruessner JC, Hellhammer DH, Kirschbaum C. Burnout, perceived stress, and cortisol responses to awakening. Psychosom Med. 1999; 61:197–204. [PubMed: 10204973]

R Development Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2010. Available at: http://www.R-project.org/.

Randolph JF, Sowers M, Gold EB, Mohr BA, Luborsky J, Santoro N, McConnell DS, Finkelstein JS, Korenman SG, Matthews KA, Sternfeld B, Lasley BL. Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status. J Clin Endocrinol Metab. 2003; 88:1516–1522. [PubMed: 12679432]

Riad-Fahmy D, Read GF, Walker RF, Griffiths K. Steroids in saliva for assessing endocrine function. Endocr Rev. 1982; 3:367–395. [PubMed: 6217969]

Scott EM, McGarrigle HHG, Lachelin GCL. The increase in plasma and saliva cortisol levels in pregnancy is not due to the increase in corticosteroid-binding globulin levels. J Clin Endocrinol Metab. 1990; 71:639–644. [PubMed: 2394772]

Slag MF, Ahmed M, Gannon MC, Nuttall FQ. Meal stimulation of cortisol secretion: a protein-induced effect. Metabolism. 1981; 30:1104–1108. [PubMed: 6270500]

Thornburg J, Spielvogel H, Vitzthum V. Salivary progesterone as a proxy for serum progesterone: reports of its death have been greatly exaggerated. Am J Hum Biol. 2008; 20:236.

Trumble BC, Brindle E, Kupsik M, O'Connor KA. Responsiveness of the reproductive axis to a single missed evening meal in young adult males. Am J Hum Biol. 2010; 22:775–781. [PubMed: 20721980]

Umeda T, Hiramatsu R, Iwaoka T, Shimada T, Miura F, Sato T. Use of saliva for monitoring unbound free cortisol levels in serum. Clin Chim Acta. 1981; 110:245–253. [PubMed: 6261989]

Vining R, McGinley R, Symons R. Hormones in saliva: mode of entry and consequent implications for clinical interpretation. Clin Chem. 1983; 29:1752–1756. [PubMed: 6225566]

Vitzthum VJ. Evolutionary models of women's reproductive functioning. Ann Rev Anthropol. 2008; 37:53–73.

Vitzthum VJ. The ecology and evolutionary endocrinology of reproduction in the human female. Am J Phys Anthropol. 2009; 140:95–136. [PubMed: 19890865]

Vitzthum VJ, Bentley GR, Spielvogel H, Caceres E, Thornburg J, Jones L, Shore S, Hodges KR, Chatterton RT. Salivary progesterone levels and rate of ovulation are significantly lower in poorer than in better-off urban-dwelling Bolivian women. Hum Reprod. 2002; 17:1906–1913. [PubMed: 12093859]

Vuorento T, Lahti A, Hovatta O, Huhtaniemi I. Daily measurements of salivary progesterone reveal a high rate of anovulation in healthy students. Scand J Clin Lab Invest. 1989; 49:395–401. [PubMed: 2740829]

Wang DY, Knyba RE. Salivary progesterone: relation to total and non-protein-bound blood-levels. J Steroid Biochem Mol Biol. 1985; 23:975–979.

West, B.; Welch, K.; Galecki, A. Linear mixed models: a practical guide using statistical software, Vol. 19. Boca Raton: Champan & Hall/CRC; 2007.

Wirth MM, Meier EA, Fredrickson BL, Schultheiss OC. Relationship between salivary cortisol and progesterone levels in humans. Biol Psychol. 2007; 74:104–107. [PubMed: 16904811]

Wong YF, Mao K, Panesar NS, Loong EPL, Chang AMZ, Mi ZJ. Salivary estradiol and progesterone during the normal ovulatory menstrual cycle in Chinese women. Eur J Obstet Gynecol Reprod Biol. 1990; 34:129–135. [PubMed: 2303146]

Wood P. Salivary steroid assays: research or routine? Ann Clin Biochem. 2009; 46:183–196. [PubMed: 19176642]

Yaneva M, Mosnier-Pudar H, Dugué M-A, Grabar S, Fulla Y, Bertagna X. Midnight salivary cortisol for the initial diagnosis of Cushing's syndrome of various causes. J Clin Endocrinol Metab. 2004; 89:3345–3351. [PubMed: 15240613]

Young EA, Breslau N. Saliva cortisol in posttraumatic stress disorder: a community epidemiologic study. Biol Psych. 2004; 56:205–209.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

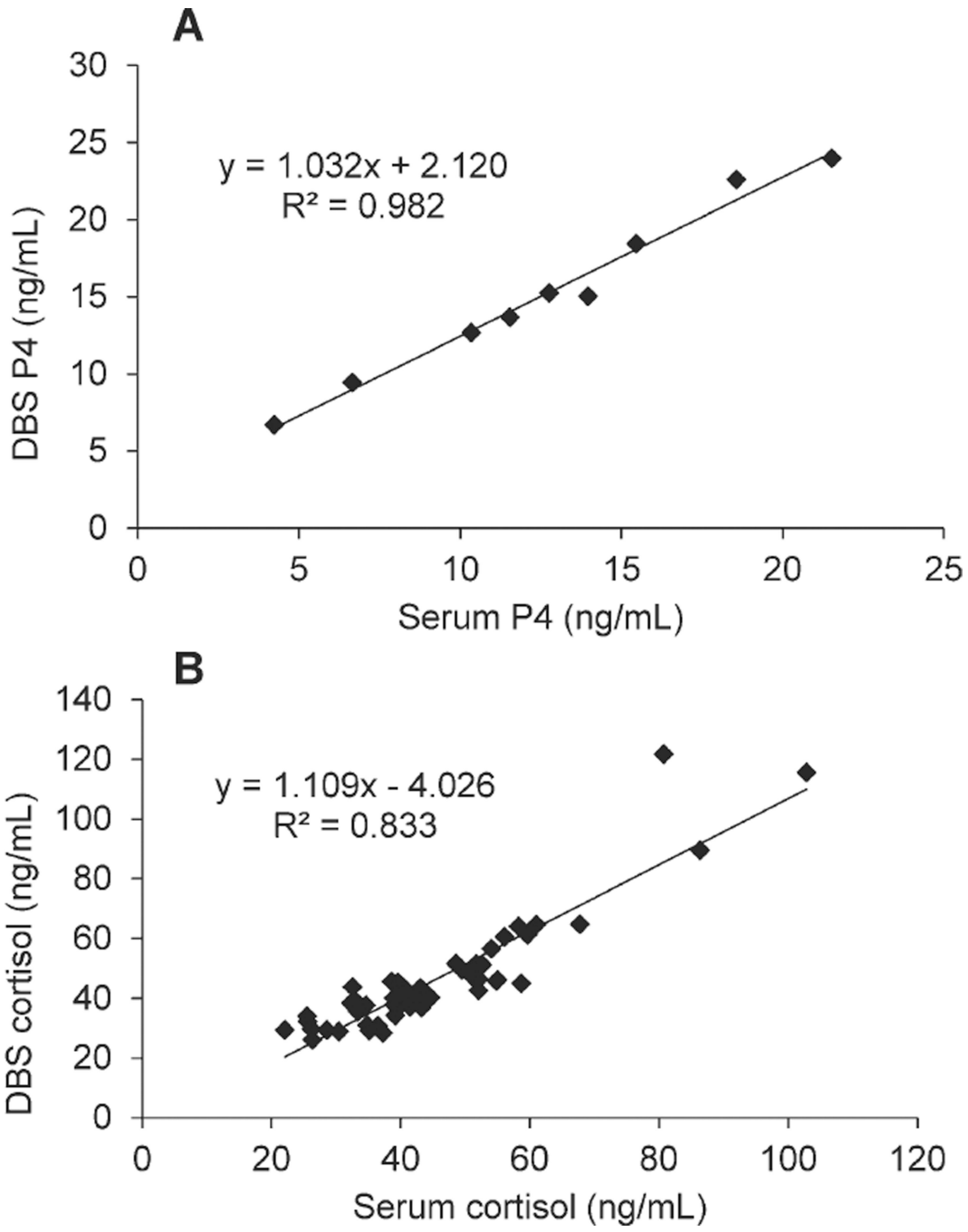

Fig. 1.

Correlation between (**A**) serum and DBS P4 concentrations (*N* = 9 sets; unpublished data) and (**B**) between serum and DBS cortisol concentrations (*N* = 51 sets; unpublished data) in matched specimens.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

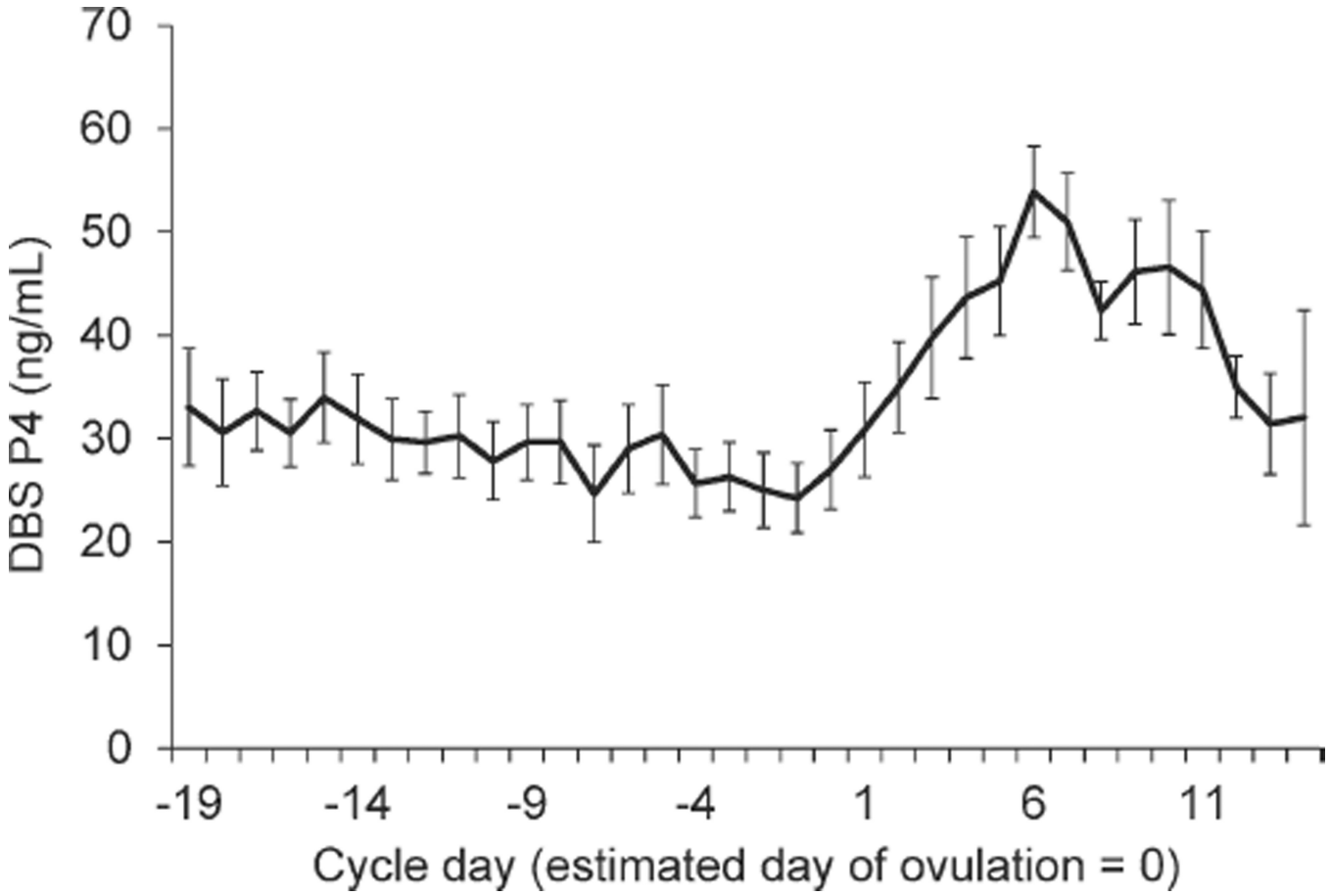

Fig. 2.

Mean and SE of DBS P4 across a menstrual cycle aligned with an estimated day of ovulation from nine women who participated in every-day sampling ($N = 9$).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

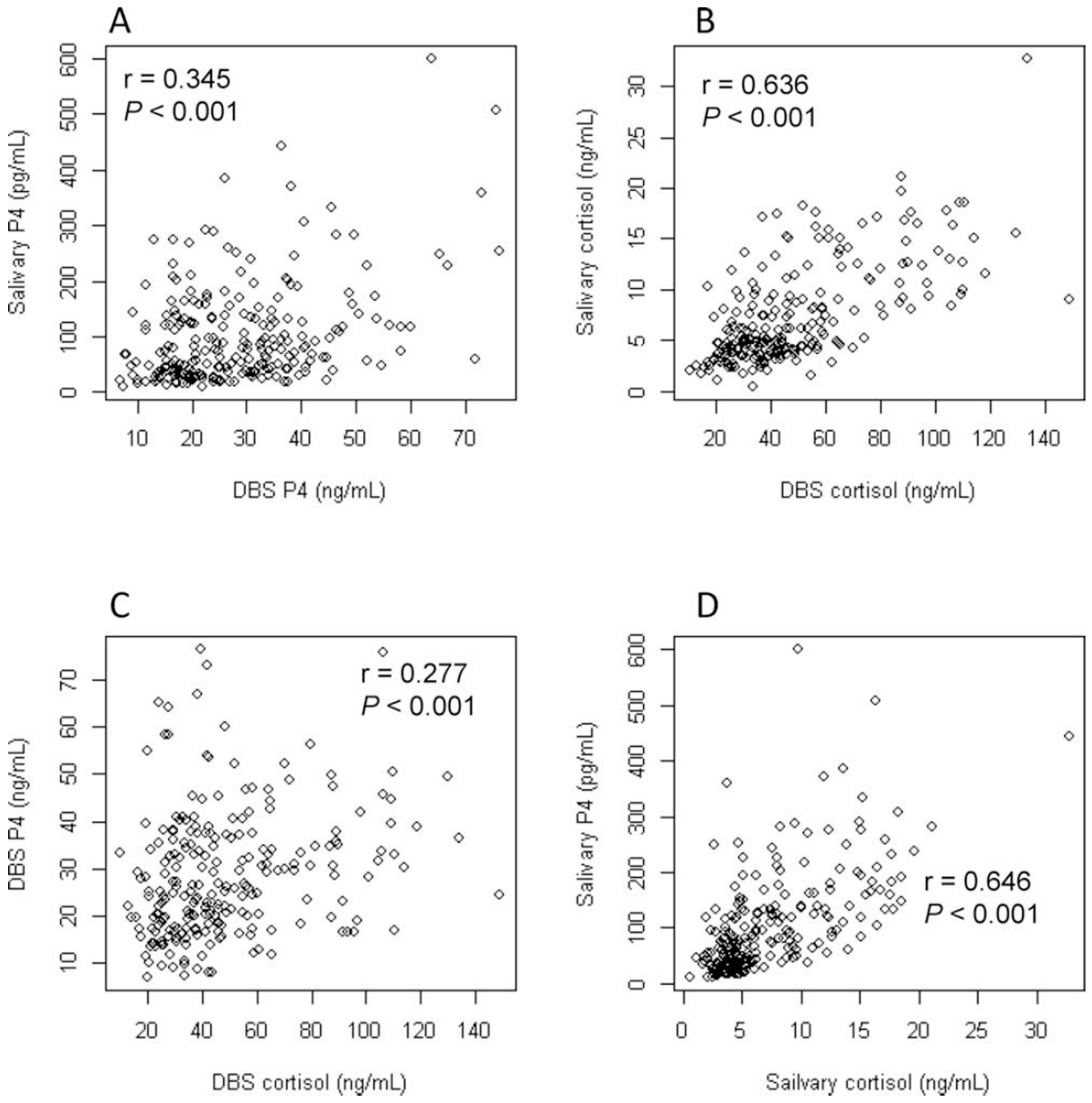

Fig. 3.

Correlation between (**A**) concentration of salivary and DBS P4, (**B**) salivary and DBS cortisol, (**C**) DBS P4 and cortisol, and (**D**) salivary P4 and cortisol (N = 232).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

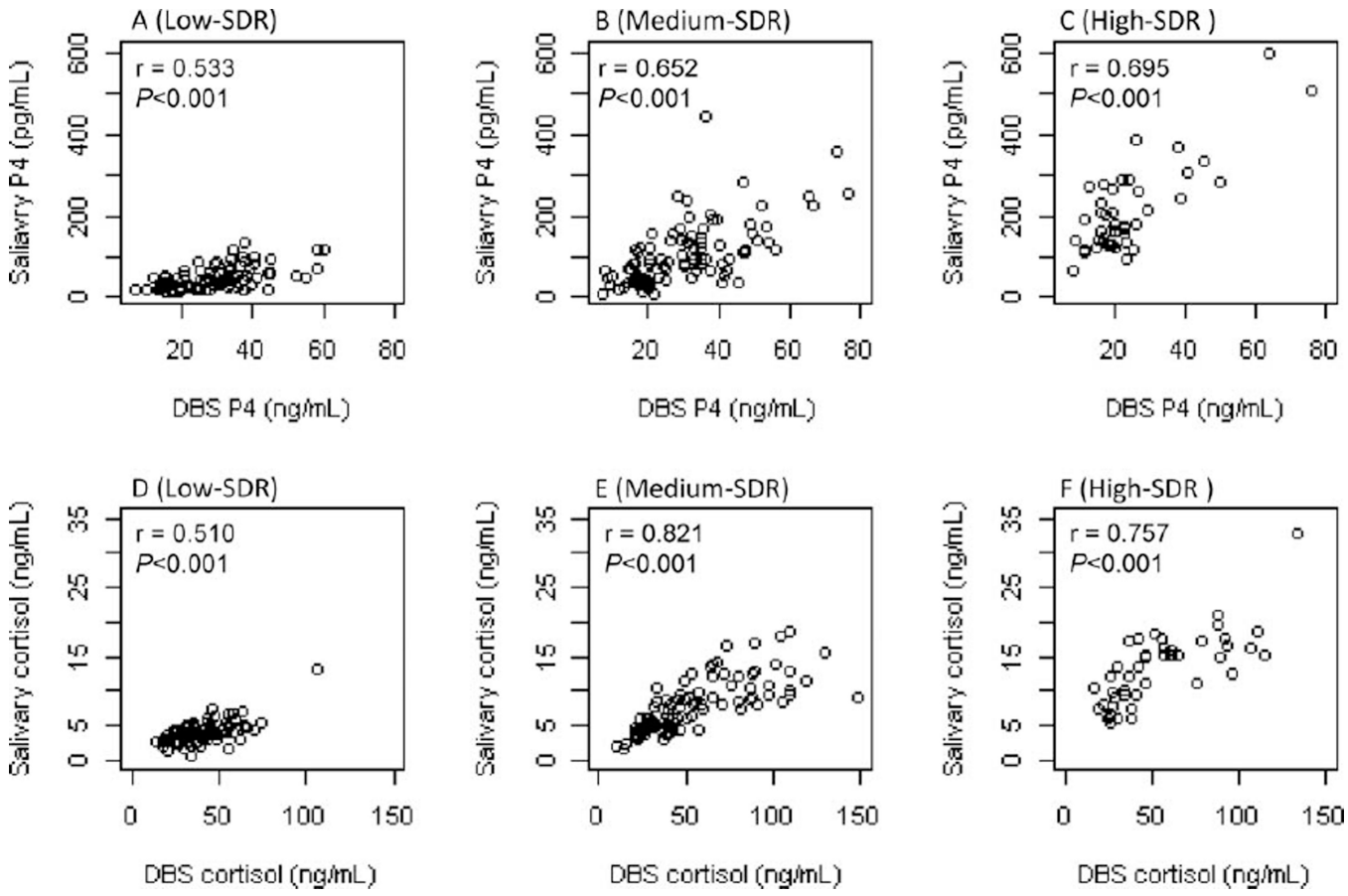

Fig. 4.

Correlation between concentration of salivary and DBS P4 in (**A**) low- (*N* = 23 women), (**B**) medium- (*N* = 24), and (**C**) high-SDR (*N* = 11) for P4, and of salivary and DBS cortisol in (**D**) low- (*N* = 22), (**E**) medium- (*N* = 25), and (**F**) high-SDR (*N* = 11) for cortisol. Each woman gave four sets of matched specimens.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

TABLE 1

Characteristics of the participants (N = 58) and paired blood and saliva specimens (N = 232 sets)[a]; mean ± SD and [ranges], proportions (N), or median (1Q, 3Q)

Attributes	Caucasian; *N* = 43; *N* = 172 sets	Japanese; *N* = 15; *N* = 60 sets	*P*[b]	Total; *N* = 58; *N* = 232 sets
Participant characteristics				
Age (years)	23.4 ± 4.2 [18–33]	25.9 ± 4.4 [19–33]	0.068	24.0 ± 4.4 [18–33]
Height (cm)	166.7 ± 6.2 [155.5–180.7]	160.7 ± 5.1 [153.7–169.0]	<0.001	165.1 ± 6.5 [153.7–180.7]
Weight (kg)	64.3 ± 12.1 [47.8–107.5]	52.7 ± 5.2 [44.0–65.8]	<0.001	61.3 ± 11.9 [44.0–107.5]
BMI (kg m^{-2})	23.1 ± 4.0 [17.0–38.8]	20.4 ± 2.0 [15.9–23.7]	0.001	22.4 ± 3.7 [15.9–38.8]
WC (cm)	73.4 ± 9.3 [61.8–114.5]	67.0 ± 3.1 [62.8–71.7]	<0.001	71.7 ± 8.6 [61.8–114.5]
HC (cm)	100.0 ± 8.9 [88.6–129.3]	92.2 ± 4.2 [83.6–99.8]	<0.001	97.9 ± 8.6 [83.6–129.3]
Percent body fat	30.9 ± 6.7 [16.7–52.5]	28.6 ± 4.1 [19.8–34.6]	0.089	30.2 ± 6.2 [16.7–52.5]
MUAC (cm)	27.9 ± 4.0 [19.4–42.4]	25.5 ± 2.6 [19.5–29.4]	0.010	27.3 ± 3.8 [19.4–42.4]
Biceps (mm)	19 ± 9 [4–48]	17 ± 5 [8–29]	0.435	18 ± 8 [4–48]
Triceps (mm)	26 ± 10 [9–57]	25 ± 5 [15–36]	0.885	26 ± 9 [9–57]
Specimen characteristics				
Time of day at sampling				
Early morning[c]	21% (*N* = 36)	30% (*N* = 18)	0.369	23% (*N* = 54)
Late morning[d]	41% (*N* = 71)	35% (*N* = 21)		40% (*N* = 92)
Afternoon[e]	38% (*N* = 65)	35% (*N* = 21)		37% (*N* = 86)
Hormone concentrations				
DBS P4 (ng mL^{-1})	27.6 (19.4, 37.3)	23.9 (17.2, 34.3)	0.132	26.1 (18.2, 36.4)
Salivary P4 (pg mL^{-1})	74.7 (40.9, 135.0)	82.2 (31.6, 136.4)	0.732	74.7 (37.7, 135.0)
DBS cortisol (ng mL^{-1})[f]	42.6 (30.1, 58.3)	41.4 (28.0, 59.7)	0.739	41.6 (29.8, 58.7)
Salivary cortisol (ng mL^{-1})	5.4 (4.0, 10.4)	4.9 (4.0, 8.1)	0.360	5.3 (4.0, 9.5)

WC, waist circumference; HC, hip circumference; MUAC, mid-upper arm circumference; DBS, dried blood spot.

[a] Four sets of matched (DBS and saliva) specimens were collected from each participant.

[b] By *t* test for age and anthropometric variables, by Fisher's Exact test for time of day at sampling, or by Wilcoxon's rank sum test for hormone concentrations. Repeated measures were not corrected for in these analyses.

[c] 5–9 am.

[d] 9 am–noon.

[e] Noon–6 pm.

[f] *N* = 171 for Caucasian, *N* = 60 for Japanese women, and *N* = 231 for total.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 2

Median (1Q, 3Q) of P4 and cortisol concentrations in DBS and saliva specimens collected at different times of day

	DBS P4 (ng mL^{-1})	Salivary P4 (pg mL^{-1})	DBS cortisol (ng mL^{-1})	Salivary cortisol (ng mL^{-1})
Early morning[a] (N = 54)[b]	28.9 (20.5, 37.7)	121.0 (57.7, 167.5)	61.4 (48.7, 89.1)	9.4 (6.2, 13.5)
Late morning[c] (N = 92)	29.5 (18.0, 37.7)	72.1 (40.2, 132.3)[d]	42.0 (33.5, 59.3)[e]	5.4 (4.2, 9.4)[e]
Afternoon[f] (N = 86)	23.9 (17.3, 32.9)	58.3 (29.1, 119.6)[e]	30.8 (25.6, 43.6)[e]	4.3 (3.3, 6.1)[e]
Total (N = 232)[g]	26.1 (18.2, 36.4)	74.7 (37.7, 135.0)	41.6 (29.8, 58.7)	5.3 (4.0, 9.5)

[a] 5–9 am.

[b] N = 53 for DBS cortisol.

[c] 9 am–noon.

[d] $P < 0.05$ vs. early morning, by Wilcoxon's rank sum test.

[e] $P < 0.001$ vs. early morning, by Wilcoxon's rank sum test.

[f] Noon–6 pm.

[g] N = 231 for DBS cortisol.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 3

Effect of diurnal variation and ethnicity on salivary hormones while controlling for DBS hormone level (each hormone modeled separately). Beta coefficients (SE) of multiple linear fixed-effects (LFE) model analyses and linear mixed-effects (LME) model analyses are shown for each hormone (N = 231)

	Salivary P4 (pg mL^{-1})		Salivary cortisol (ng mL^{-1})	
Predictor	**LFE**	**LME**	**LFE**	**LME**
DBS hormone[a]	2.654 (0.417)***	3.856 (0.338)***	0.109 (0.011)***	0.087 (0.009)***
Time of day at sampling				
Late morning[b,c]	−21.62 (14.50)	−16.42 (11.49)	−0.453 (0.653)	−0.266 (0.527)
Afternoon[b,d]	−31.47 (14.70)*	−11.65 (12.19)	−1.426 (0.725)	−1.596 (0.603)**
Japanese ethnicity[e]	−5.324 (13.40)	−1.236 (24.02)	−1.002 (0.574)	−1.006 (1.004)
BMI	−2.001 (1.580)	−2.244 (2.387)	0.017 (0.068)	0.023 (0.119)

[a] DBS P4 (ng mL^{-1}) or DBS cortisol (ng mL^{-1}) for salivary P4 and cortisol models, respectively.

[b] vs. early morning (5–9 am).

[c] 9 am–noon.

[d] Noon–6 pm.

[e] vs. Caucasian.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

TABLE 4

Saliva-to-DBS hormone ratio (SDR) categories of P4 and cortisol for each participant. DBS hormone (P4 or cortisol) concentration, time of day, ethnicity, and BMI were adjusted for prior to categorization by SDR. Table cells are numbers of participants (and number of Caucasian/Japanese participants) in the category

		Cortisol (*N*)			
		Low-SDR[a]	**Medium-SDR[b]**	**High-SDR[c]**	**Total**
P4 (*N*)	Low-SDR[a]	16[d] (11/5)	7[d] (6/1)	0[d] (0/0)	23 (17/6)
	Medium-SDR[b]	5[d] (4/1)	17[d] (13/4)	2[d] (2/0)	24 (19/5)
	High-SDR[c]	1[d] (1/0)	1[d] (0/1)	9[d] (6/3)	11 (7/4)
	Total	22 (16/6)	25 (19/6)	11 (8/3)	58 (43/15)

[a]Participants whose residuals of salivary hormone levels with the LFE models were negative for all four matched specimen sets.

[b]Participants whose residuals of salivary hormone levels with the LFE models were both negative and positive.

[c]Participants whose residuals of salivary hormone levels with the LFE models were positive for all four matched specimen sets.

[d]Fisher's exact test; $P < 0.001$.