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Brief report

# Relationship between salivary cortisol and progesterone levels in humans

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**Abstract**

In four studies, each with multiple hormone assessments before and after positive emotion-arousing laboratory manipulations, salivary progesterone positively correlated with salivary cortisol in men and women taking hormonal contraceptives but not in freely cycling women. This is consistent with the idea that progesterone in men is largely adrenal in origin, whereas in women its sources are both ovarian and adrenal. In addition, bi-partial correlations revealed that change in cortisol was positively related to change in progesterone levels; this effect was stronger in men than in women. These findings suggest that progesterone is released from the adrenal along with cortisol in humans, due to general adrenal activation and/or possibly as an additional negative feedback mechanism to down-regulate the stress response.


*Keywords:* Human; Sex differences; Hormones; Hormonal contraceptives; Oral contraceptives; Progesterone; Cortisol; Bi-partial correlation; HPA axis

## 1. Introduction

Though progesterone (PROG) is generally known as a gonadally released reproductive hormone, it is also produced in the brain and by the adrenal gland, where PROG is an indirect precursor to cortisol (CORT) (Baulieu et al., 2001). Like CORT, PROG is released in response to adrenocorticotropin hormone (ACTH) (Genazzani et al., 1998), and levels peak in the morning and decline over the course of the day (Groschl et al., 2003). Male mammals produce considerable amounts of PROG; for example, men have circulating levels of unbound PROG roughly equal to women in the early follicular phase (Schultheiss et al., 2003). It is likely that the adrenals are the main source of PROG in males. In females, both the ovary and the adrenal contribute to circulating PROG levels.

There is considerable evidence that PROG has functions beyond female reproduction. For example, PROG and its metabolites have anxiolytic and sedative properties, most likely via action of the metabolites at GABA receptors (Paul and Purdy, 1992; Soderpalm et al., 2004). In rodents, PROG and allopregnanolone down-regulate HPA axis responses to stress (Guo et al., 1995; Patchev et al., 1994, 1996). Also, studies in both humans and other animals have found increases in PROG and its metabolite, allopregnanolone, in response to stress (Barbaccia et al., 1996; Genazzani et al., 1998; Girdler et al., 2001; Klatzkin et al., 2006; Purdy et al., 1991) and other motivation/emotion-arousing stimuli (Schultheiss et al., 2004). These rapid PROG responses are likely to be adrenal in origin. PROG and allopregnanolone have also been implicated in affective disorders (e.g., Brambilla et al., 2004, 2005; Eser et al., 2006).

Thus, PROG has important functions outside of reproduction. Both PROG and CORT play roles in affect and stress and could be important factors in human health. Despite the importance of these hormonal systems, however, the relationship between PROG and CORT has not been characterized in humans. Here we report strong relationships between salivary CORT and PROG in men and in women suppressing ovarian function with hormonal contraceptives, but not in cycling women. These effects replicated across four studies of varying design.

## 2. Methods

Participants in all studies were University of Michigan students recruited via introductory psychology subject pool and through flyers. All studies were approved by the University of Michigan Institutional Review Board, and all participants provided informed consent. Participants received subject pool credit or payment of US$ 10 for each hour of participation.

Four studies were conducted to investigate hypotheses concerning positive emotion, affiliation, and the relationship between these constructs and PROG and CORT. As a side hypothesis, we investigated relationships between CORT and PROG in all four studies. In studies 1 and 2, participants (study 1: $N = 59$, 39 women; study 2: $N = 89$, 39 women) were randomly assigned to view one of

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four 30-min film clips chosen to arouse social emotional–motivational states (power/dominance; hope for closeness; fear of rejection; neutral control). Participants provided saliva samples at the beginning of the 2-h session (T1), 10 min (T2) and 30 min (T3) following film presentation, and completed other instruments measuring emotion and personality traits. Sessions were run between 10:00 and 17:00 (study 1) and 12:00 and 18:00 (study 2). Effects of film condition on PROG and testosterone in study 1 have been published previously (Schultheiss et al., 2004). Relationships between film condition, motivation, and hormones in study 2 will be published in a separate report (Wirth and Schultheiss, in preparation). Studies 3 and 4 were designed to arouse positive emotion through positive social interaction. In study 3 ($N = 143$, 77 women), participants underwent a mood-induction paradigm in which they were randomly assigned to one of four conditions: exchange of positive or neutral memories with a same-sex partner, or generation of positive or neutral memories alone. Participants then completed questionnaires and played a card game requiring cooperation with their partners. Saliva samples were collected at the beginning (T1) and end (T2) of the 1-h session. In study 4 ($N = 82$, 41 women), participants, in same-sex pairs, engaged in a cooperative card game for 30 min. Saliva samples were collected at the beginning of the session (T1), immediately before the card game (at 30 min; T2), and at the end of the session (at 90 min; T3). All sessions in studies 3 and 4 took place between 12:00 and 19:00. Female participants in all four studies reported menstrual cycle information, as well as whether they were using hormonal contraceptives (10, 17, 19, and 10 women, respectively). No participants in any study reported using hormonally active medications other than hormonal contraceptives.

Salivary measurements are an excellent non-invasive way to assess unbound steroid hormones in humans (Riad-Fahmy et al., 1983). Saliva samples were collected and processed using the same procedure described in (Wirth et al., 2006). Salivary CORT and PROG levels were determined by solid-phase $^{125}$I radioimmunoassays (Coat-A-Count, Diagnostic Products Corp., Los Angeles). Hormones were measured using 400 μl saliva samples in combination with water-diluted standards (analytical range: CORT, 0.5–50 ng/ml; PROG, 5–400 pg/ml) and overnight incubation at room temperature. Mean intra-assay coefficients of variation (CV) for CORT assays over the four studies were 5.93; 4.34; 5.17; 4.09. Mean CVs for PROG assays were 11.73; 10.34; 11.47; 7.89. Pools of saliva collected from a number of volunteers at 8:00 and 20:00 showed expected circadian variation in CORT (averages 4.39 and 0.87 ng/ml). Inter-assay CVs for these pools averaged 5.85% for CORT and 16.5% for PROG. Mean CORT and PROG assay sensitivities (B0–3S.D.) were 0.017 ng/ml and 1.89 pg/ml, respectively.

## 3. Results

CORT and PROG concentrations by sex and time point for each study are shown in Table 1. CORT and PROG tended to decrease over the course of each study (as expected due to circadian fluctuation).

In all four studies, salivary PROG had a significant positive correlation with CORT at almost all time points in men, and at no time points in women (Table 2). CORT and sometimes PROG distributions were skewed; however, a log-transformation of the data did not alter the correlation relationships reported here. PROG and CORT positively correlated in women using hormonal contraceptives in two of the four studies (Table 2).

Combining results across all studies, PROG and CORT averaged across all measurements were strongly positively correlated in men ($N = 176$; $R = 0.42$; $P < 0.00001$) and in women using hormonal contraceptives ($N = 60$; $R = 0.42$; $P = 0.0008$) but not in women not taking hormonal contraceptives ($N = 137$; $R = -0.03$; $P = 0.71$). In the latter group, in a regression of average CORT on average PROG, using number of days since onset of last menstruation (CYCLEDAY) did not change the results. In fact, the main effect of CYCLEDAY on PROG only reached a trend level, $F(1, 124) = 1.86$, $P = 0.07$. It should be noted that CYCLEDAY is a crude measure of menstrual status based on self-report; a more controlled study design would be necessary to sort out effects of menstrual phase on the relationship between the two hormones.

In the combined analysis, time of day (TIME) negatively predicted CORT ($R = -0.19$, $P = 0.0005$) but had no relationship with PROG. In regression analyses of PROG on CORT (or vice versa) including TIME as a factor, TIME did not interact with PROG in predicting CORT. The lack of effects of TIME are probably due to the fact that in all but one study, sessions were restricted to the afternoon, thus avoiding the morning period of rapid decline in steroid hormones.

Returning to a study-by-study analysis, bi-partial correlation analysis (Cohen and Cohen, 1983) was used to estimate the correspondence between PROG changes and CORT changes. Bi-partial correlations represent the co-variation between CORT and PROG residuals at a given time point after controlling for each hormone's levels at the prior time point. These analyses, shown in Table 3, revealed that CORT and PROG co-varied positively between most adjacent sampling time points in men in all four studies, and in women between some time points in some of the studies.

In study 1, for which testosterone data were available, PROG and testosterone correlated in men only at T2 ($R = 0.59$, $P = 0.007$), and in women only at T1 ($R = 0.42$, $P = 0.009$). Bi-partial correlations revealed no relationship between changes in

Table 1
CORT (ng/ml) and PROG (pg/ml) by sex and sampling time point: mean (S.D.)

| | Study 1 ($N = 59$, 39 women) | | Study 2 ($N = 89$, 39 women) | | Study 3 ($N = 143$, 77 women) | | Study 4 ($N = 82$, 41 women) | |
|---|---|---|---|---|---|---|---|---|
| | CORT | PROG | CORT | PROG | CORT | PROG | CORT | PROG |
| M | | | | | | | | |
| T1 | 2.1 (0.8) | 25.2 (6.4) | 3.1 (0.3) | 21.6 (9.4) | 2.9 (1.7) | 21.3 (18.6) | 2.5 (1.9) | 17.6 (6.8) |
| T2 | 1.6 (0.9) | 21.9 (7.0) | 2.1 (0.2) | 16.6 (5.7) | 2.3 (1.1) | 17.7 (21.9) | 2.3 (1.4) | 16.8 (6.3) |
| T3 | 1.4 (0.5) | 21.0 (6.7) | 1.8 (0.2) | 15.9 (6.2) | N/A | N/A | 1.6 (0.7) | 15.5 (5.8) |
| F | | | | | | | | |
| T1 | 3.0 (2.0) | 38.1 (40.1) | 2.2 (0.2) | 23.4 (22.0) | 2.6 (1.6) | 21.1 (15.6) | 2.2 (1.0) | 36.4 (40.7) |
| T2 | 2.3 (1.4) | 30.5 (26.7) | 1.6 (0.2) | 19.1 (19.1) | 1.9 (1.0) | 17.6 (15.5) | 1.9 (0.9) | 31.8 (33.7) |
| T3 | 1.8 (1.1) | 32.5 (32.1) | 1.4 (0.1) | 20.4 (24.1) | N/A | N/A | 1.4 (0.6) | 29.3 (32.4) |

M: males; F: females.

Table 2
Correlations between CORT and PROG: *R*(*P*)

| | Study 1 (*N* = 59, *W* = 29, W-HC = 10) | Study 2 (*N* = 89, *W* = 22, W-HC = 17) | Study 3 (*N* = 143, *W* = 58, W-HC = 19) | Study 4 (*N* = 82, *W* = 31, W-HC = 10) |
|---|---|---|---|---|
| M | | | | |
| T1 | 0.23 (NS) | 0.52 (0.0001) | 0.51 (<0.0001) | 0.50 (0.0009) |
| T2 | 0.78 (<0.0001) | 0.60 (<0.0001) | 0.45 (0.0002) | 0.40 (0.010) |
| T3 | 0.63 (0.003) | 0.49 (0.0003) | N/A | 0.39 (0.012) |
| W | | | | |
| T1 | −0.03 (NS) | 0.01 (NS) | −0.09 (NS) | 0.27 (NS) |
| T2 | 0.05 (NS) | −0.14 (NS) | −0.09 (NS) | 0.10 (NS) |
| T3 | 0.03 (NS) | −0.07 (NS) | N/A | 0.07 (NS) |
| W-HC | | | | |
| T1 | 0.24 (NS) | 0.52 (0.04) | 0.54 (0.008) | 0.15 (NS) |
| T2 | −0.14 (NS) | 0.63 (0.01) | 0.35 (0.10) | 0.37 (NS) |
| T3 | 0.01 (NS) | 0.61 (0.01) | N/A | 0.10 (NS) |

M: men; W: women not using hormonal contraceptives; W-HC: women on hormonal contraceptives; T1: first time point, etc.

Table 3
CORT–PROG bi-partial correlations between adjacent sampling time points: *R*(*P*)

| | Study 1 | Study 2 | Study 3 | Study 4 |
|---|---|---|---|---|
| M | | | | |
| T1–T2 | 0.67 (0.001) | 0.40 (0.003) | 0.44 (0.0002) | 0.52 (0.0007) |
| T2–T3 | 0.27 (0.25) | 0.38 (0.007) | N/A | 0.45 (0.004) |
| F | | | | |
| T1–T2 | 0.11 (0.51) | 0.15 (0.36)[a] | −0.13 (0.27) | 0.48 (0.002) |
| T2–T3 | 0.31 (0.06) | 0.53 (0.0006) | N/A | 0.26 (0.09) |

M: males; F: females.
[a] Significant positive bi-partial correlation in women using hormonal contraceptives (*N* = 17 in study 2).

PROG and changes in testosterone in women or men. CORT and testosterone correlated in men only at T2 ($R = 0.54$, $P = 0.015$) and never in women.

## 4. Discussion

In four studies with a total of 373 participants, CORT and PROG were strongly positively correlated in men, but only in women who were using hormonal contraceptives. In addition, bi-partial correlations revealed that change in cortisol was positively related to change in progesterone levels. This effect was more consistent in men than in women.

These findings make sense assuming that circulating PROG comes primarily from the adrenal in men and from the ovary in cycling women. Adrenal PROG release, along with CORT, is likely stimulated by ACTH (Genazzani et al., 1998). However, changes in adrenal PROG may be "drowned out" in cross-sectional assessments by ovarian PROG in cycling women, which is released in response to gonadotropin pulses.

PROG, like CORT and other steroid hormones, peaks in the morning and declines throughout the day (Groschl et al., 2003). However, since the majority of participants were run in the late afternoon, time of day had a weak correlation with CORT, did not impact PROG, and did not affect the relationship between the two hormones. The fact that levels of both hormones were dropping on average over the course of the sessions may partially account for the significant bi-partial correlations. However, one of the three conditions in study 2 led to a CORT increase relative to baseline; post-manipulation PROG levels were also highest in this group (Wirth and Schultheiss, in preparation). This suggests that the relationship between the two hormones is not merely a side effect of circadian decline.

Though the relationships between PROG and CORT may simply reflect the fact that adrenal activity in general produces multiple hormones, note that testosterone did not show a strong relationship with PROG in either sex, even though PROG is an indirect precursor to testosterone as well as CORT.

PROG, via its metabolite allopregnanolone, exerts sedative, anxiolytic, and HPA-axis-suppressing effects (Guo et al., 1995; Patchev et al., 1994, 1996; Paul and Purdy, 1992). It has been suggested that PROG and allopregnanolone are released during stress in order to down-regulate HPA axis activity and anxiety (Barbaccia et al., 1996). Close relationships between PROG and CORT could reflect a functional relationship such that PROG is released from the adrenal along with CORT in order to help down-regulate the HPA axis. This may be true during stress as well as under basal conditions. Further research should examine whether the CORT–PROG relationships reported here are altered in affective disorders. Future studies should could also investigate whether, due to the stronger influence of the ovary in determining circulating PROG levels, PROG and its anxiolytic metabolites are less stress-responsive in women than in men, and whether this is a contributing factor in women's greater risk for affective disorders.

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