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*Original Research Article*

# Progesterone and Estrogen Responsiveness to Father-Toddler Interaction

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**Objectives:** We assessed the responsiveness of salivary progesterone (P4) and estradiol (E2) to father-child interaction, including testing for differences in short-term hormonal change based on paternal characteristics. We also predicted that P4 exposure during the study period would relate positively to post-interaction paternal mood.

**Methods:** We conducted an in-home intervention study in which fathers ($n = 44$) played with their toddlers. Subjects provided saliva samples before interacting with their children, with additional collections 40 and 70 min later.

**Results:** E2 did not significantly change over the study period ($P > 0.4$). P4 declined significantly from baseline to 40 min ($P < 0.05$) and 70 min ($P < 0.001$). Men reporting that the interaction made them feel very happy/relaxed had greater P4 exposure from baseline through 70 min (area under the curve) compared with men reporting less positive post-interaction mood ($P < 0.05$). This relationship persisted after controlling for cortisol. Men's % decrease in P4 (baseline to 40 min) was significantly greater if they had an infant ($P < 0.05$), while fathers' % decline in E2 (baseline to 70 min) was larger if they had more children ($P < 0.05$).

**Conclusions:** These results require replication but could indicate that grouping fathers with different levels of experience obscures meaningful variation in hormonal responses to child interaction. Our findings appear consistent with the effects of P4 as a mood enhancer and suggest future research should explore the possible role of P4 as hormonal mechanism that could reinforce or facilitate paternal investment. Am. J. Hum. Biol. 25:491–498, 2013. © 2013 Wiley Periodicals, Inc.

There is a growing body of psychobiological literature on the ways in which men's hormonal profiles covary with their romantic partnering and parenting status, as, for example, fathers have lower testosterone (T) than other men in some cultural settings (Gray and Campbell, 2009). Lower T is posited to enhance a father's focus on the needs of his partner and dependent offspring and orient him away from social domains related to male-male competition and mating effort (Gettler, 2010; Gray and Anderson, 2010; van Anders et al., 2011; Wingfield et al., 1990). Relationships generally consistent with this model have increasingly been observed in human males, e.g. paternal T is lower in conjunction with greater direct childcare (Alvergne et al., 2009; Gettler et al., 2011b; Muller et al., 2009). However, while this T-oriented model has gained traction, few studies have focused on other hormones that might have implications for human paternal investment (but see Feldman et al., 2010; Gettler et al., 2012b; Gray et al., 2007).

Two under-explored hormones that could function in such roles are estradiol (E2) and progesterone (P4), which have long occupied a prominent position in research on female physiology because of their importance to the ovulatory cycle and pregnancy (Ellison, 2001). E2 and P4 also influence the expression of maternal behavior in many mammalian species (Numan and Insel, 2003), yet surprisingly little is known about their psychobiological implications for human parenting, especially in fathers. In a recent study, elevated E2 was linked to desire to have more children in nulliparous women (Law Smith et al., 2011), and elsewhere mothers with less of a decline in the E2 to P4 ratio from pregnancy to post-partum were found to have higher post-partum attachment to their infants (Fleming et al., 1997). In a separate study, neither E2 nor P4 were significantly correlated to maternal behavior in the early post-partum (Fleming et al., 1987).

Studies focusing on the psychobiological implications of P4 and E2 in human males are relatively scant, despite the fact that men and non-pregnant women (in the follicular phase) have broadly similar circulating levels of P4, which is an important steroidogenic precursor as well as a bio-active steroid in its own right (Oettel and Mukhopadhyay, 2004). Male P4 production occurs both in the adrenals and the testes, with studies varying in the extent to which circulating P4 is more strongly linked to activity of the hypothalamic-pituitary-adrenal (HPA) or hypothalamic-pituitary-gonadal (HPG) axes (Genazzani et al., 1998; Kage et al., 1982; Liening et al., 2010; Wirth et al., 2007). Circulating male E2, which is primarily a product of T aromatization, serves multiple important physiological roles throughout the male life course, including contributing to the negative feedback loop regulating T production (Bagatell et al., 1994) and potentially exerting developmental effects on sex differentiation in the brain (McCarthy and Arnold, 2011), although this is debated for higher primates (Wallen, 2005).

From a cross-species perspective, there is mixed evidence for a potential role of E2 in mammalian paternal investment. In at least one murine species, it is known that conversion of T to E2 is necessary for the normal expression of paternal behavior (Trainor and Marler,

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2002; Trainor et al., 2003), yet lower E2 brain receptor expression correlates to higher paternal care in other rodents (Cushing and Wynne-Edwards, 2006). Results among non-human primates are also variable, as fathers' peak caregiving coincided with E2 decline in one New World monkey species (Nunes et al., 2001), while, in a separate species, experienced fathers' E2 rose in the months leading to parturition (Ziegler et al., 2004).

Among human males, in the only study of its kind to date, it was found that fathers had higher salivary E2 relative to non-father controls, and the percentage of fathers with detectable E2 increased from the pre- to the post-partum period (Berg and Wynne-Edwards, 2001). These findings are preliminarily suggestive of direct upregulation of paternal E2 production during the early infancy period. This is noteworthy given that many fathers experience a decline in T during the early post-partum (Berg and Wynne-Edwards, 2001; Gettler et al., 2011b; Storey et al., 2000), which would be expected to lower E2 by limiting the available pool of T for aromatization. However, evidence elsewhere suggests E2 might interfere with effective fathering. In a study of male undergraduates, elevated E2 related to greater impulsive extraversion and sensation seeking (Daitzman and Zuckerman, 1980), and positive correlations between E2 and measures of aggression have also been documented (Eriksson et al., 2003; Gladue, 1991), though not in all studies (Christiansen and Winkler, 1992; O'Connor et al., 2004).

The literature on P4 and male psychobiology is also relatively sparse, and, to our knowledge, P4 has never been studied in human fathers. In rodent models, P4 was found to increase from pre- to post-partum in males of a paternal hamster species, whereas they remained stable in a closely related non-paternal species (Schum and Wynne-Edwards, 2005). Conversely, P4 was found to be lower in murine fathers compared with non-fathers (Trainor et al., 2003), and P4 receptor knockout mice showed reduced aggression towards pups with increased expression of parenting behaviors (Schneider et al., 2003). In humans, there is increasing evidence that P4 and its neuroactive derivatives have anxiety-reducing effects (Childs et al., 2010; Söderpalm et al., 2004; Wirth, 2011). For example, men administered exogenous P4 before the Trier Social Stress Test (TSST), a common short-term stress reactivity paradigm, showed lower cortisol responses and less extreme changes in negative mood following TSST (Childs et al., 2010). In addition, P4 may be implicated in the fostering of social affiliation in humans (Brown et al., 2009; Wirth, 2011; Wirth and Schultheiss, 2006). Thus, although it remains to be tested, these lines of evidence suggest that in the context of paternal care, elevated P4 could serve to enhance men's mood and/or relax fathers when they interact with their offspring and perhaps help to motivate men for future father-child engagement.

Here we seek to shed light on the role of E2 and P4 in paternal psychobiology by analyzing saliva samples collected before and after fathers (age 26.6 ± 0.3 [SD] years) played with their toddlers for 15 to 30 min in their homes, located in Metro Cebu, Philippines. We have previously shown that cortisol (CORT) declines and T remains stable during this father-child interaction protocol (Gettler et al., 2011a). Acute changes in P4 might be secondary to regulation of other downstream steroid hormones, particularly CORT, and, relatedly, P4 might also decline during this afternoon study period due to circadian biology (Gröschl et al., 2003; Kage et al., 1982; Wirth et al., 2007), whereas E2 regulation is potentially tightly linked to its precursor, T. Thus, we hypothesized that E2 ($n = 39$) would remain stable and that P4 ($n = 44$) would decrease during the father-child interaction. We also explored whether there was between-father variability in E2 and P4 changes over the study period (~40 and ~70 min) based on paternal characteristics, including: (a) their number of children; (b) whether they were a father to an infant (1 year old or less); and (c) their routine involvement in father-child play. Finally, given the growing literature connecting elevated P4 to anxiolytic and sedative effects (Wirth, 2011), we used area under the curve analyses (AUC) to test the hypothesis that men with greater P4 exposure would report higher post-interaction mood ratings.

## METHODS

### *Study population*

Data were collected in 2009 and 2010 as part of the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based birth cohort study that began in 1983–1984 (Adair et al., 2011). Men were a mean of age 26.6 ± 0.3 (SD) years at the time of data and sample collection in 2010. Socioeconomic, demographic, health and general behavioral data were collected using questionnaire-based, in-home interviews administered by Cebuano-speaking interviewers (Adair et al., 2011). This research was conducted under conditions of informed consent with human subjects clearance from the Institutional Review Boards of Northwestern University and the University of San Carlos.

### *Sample characteristics*

During the 2009 survey, 908 males of the original cohort of 1,633 liveborn males were located and interviewed. Of these men, 451 were fathers. In 2010, fathers were selected for the father-child interaction study based on living with at least one biological child, older than 1 year of age and less than 4 years of age, and the mother of that child, having no adopted or step-children, and having full data from the 2005 and 2009 CLHNS surveys. A sample of 164 met these criteria. Because of budgetary constraints and the size of the Cebu metropolitan area, sampling was restricted to 23 local barangays (neighborhoods), compared with 135 barangays in the 2009 survey, resulting in a final sample of 45 men who agreed to participate. P4 data for all time points was available for 44 subjects, while 40 subjects had full E2 data. One subject was eliminated because of E2 four SD above the sample mean. Two subjects were excluded from analyses that include CORT because of an undetectable baseline value and a baseline CORT value 7+ SD above the mean, respectively.

### *Paternal involvement in play*

Men reported routine play behavior by answering, "How often do you play with your child?" Responses were on a 5-point scale ranging from, "never" to "everyday." Men were grouped based on routinely playing rarely (once a week or less; $n = 13$), frequently (multiple times per week but not daily; $n = 10$), and everyday ($n = 21$).

### *Father-child interaction protocol*

An interviewer arrived at the subject's home in the early afternoon. Men were screened for alcohol consumption in

the previous 12 hrs and for eating, cigarette smoking, or participating in rigorous activity in the hour before the interviewer's arrival. Interviewers also noted whether men were interacting with any of their children upon arrival. Before the interview began, all other individuals, including the men's wives and other children, were asked to leave the room. In the first hour of the home visit, men were consented and the questionnaire-based interview was initiated. After this preliminary hour, men provided an initial saliva sample (see below) and then were asked to play with their child for up to 30 min. Upon completion of the interaction, interviewers proceeded with the interview process, collecting additional saliva samples at 40 and 70 min. All children were provided a gift of a medium-sized plastic ball, and fathers were asked to use this toy during the interaction. Before the present study, we conducted focus groups on the subject of paternal care with fathers residing in Metro Cebu. Through these semi-structured interviews, we identified this activity (playing ball) as a common father-child interaction behavior across the age range of the present study and with both male and female offspring.

The average time for the first saliva collection was 1:46 PM ± (SD) 42.3 min. Following sample collection, interviewers set a timer for 30 min. Fathers were then asked to interact with their child. Duration of the father-child interaction averaged 24.1 ± 5.4 min (range: 15–30 min). When the 30-min timer finished, men provided a second saliva sample, an average of 41.7 ± 4.9 min after collection of the baseline sample. The third sample was collected 73.9 ± 6.9 min after baseline. All samples were transported to the University of San Carlos in Cebu City, where they were frozen at −35°C. All samples were later shipped on dry ice to Northwestern University, where they were stored at −80°C.

### *Father's post-interaction mood*

Men self-reported their mood on a 5-point scale in response to the question, "How did playing with your child make you feel?" Responses ranged from (1) very upset/nervous to (5) very happy/relaxed. A single subject reported that the interaction made him mildly upset/nervous. He was grouped with the men reporting being neither happy/upset.

### *Salivary hormonal analyses*

P4 and E2 assays were run at Northwestern University's Bass Laboratory, while T and CORT were run at Northwestern University's Laboratory for Human Biology Research. All hormones were analyzed using commercial enzyme immunoassay kits from Salimetrics (State College, PA): P4 No. 1–1502; CORT No. 1–3002; E2 No. 1–4702; T No. 1–2402. Inter-assay coefficients of variation for high and low controls were as follows: P4 4.9% and 10.7%; CORT 2.8% and 3.3%; E2 6.8% and 8.4%; T 6.4% and 7.2%.

### *Statistical analyses*

Analyses were conducted using Stata 12.1 (Stata Corporation, College Station, TX). We employed paired t-tests to assess the change in E2 and P4 during the study. Using multiple linear regression, we then predicted subjects' percentage change in P4 and E2 [baseline to saliva sample 2 (40 min) and sample 3 (70 min)] based on paternal characteristics. Using ANCOVA with post-comparison contrasts, we evaluated relationships between men's self-reported mood after the interaction and their P4 area under the curve (P4 AUC) levels. P4 AUC was calculated using the "AUC with respect to ground" formula (Pruessner et al., 2003). These models control for CORT AUC to ensure that any P4 mood effects were not simply a result of generalized adrenal activity (Wirth and Schultheiss, 2006; Wirth et al., 2007). Finally, we predicted post-interaction mood from total interaction time using ordered logistic regression. Total father-child interaction time was right-censored, as 41% of fathers played with their toddlers for the full 30 min (the imposed maximum duration). Because right-censoring of independent variables may affect general linear model statistical results (Austin and Hoch, 2004), we created dichotomous variables that categorized men according to total time and then modeled significant results using a dummy variable approach as a secondary assessment of validity. Focusing on men who did not interact for 30 min (59% of sample), we took a median split of the subjects' interaction times (range: 15–25 min), with 39% of the total sample being at or below the median of 20 min and the remaining 20% being between 21 and 25 min. By including these two dummy variables in our models, we used men who interacted for the full 30 min as the comparison group. Statistical significance was evaluated at $P < 0.05$ with relationships between $P > 0.05$ and $P < 0.10$ interpreted as a statistical trend.

*TABLE 1. Father-child interaction: Sample characteristics (n = 44)*

| | Mean | SD |
|---|---|---|
| Demographic characteristics | | |
| Age (yrs) | 26.6 | 0.3 |
| Education (highest grade) | 10.1 | 4.4 |
| Currently employed (%) | 93.2 | – |
| Urban barangay [neighborhood] (%) | 63.6 | – |
| Fatherhood characteristics | | |
| Duration of marriage (yrs) | 5.4 | 2.3 |
| No. children | 2.3 | 1.0 |
| Years as a father | 4.4 | 2.2 |
| Father of an infant (%) | 27.3 | – |
| Age of child in interaction (yrs) | 2.6 | 0.8 |
| Sex of child in interaction (% female) | 40.9 | – |
| Plays with child everyday (%) | 47.7 | – |
| Cared for child, day of study (%)[a] | 61.4 | |
| Sleep characteristics | | |
| Sleep duration prior night (hrs) | 7.9 | 1.8 |
| Slept well prior night (%)[b] | 75.0 | – |
| Unadjusted hormone values | | |
| Baseline progesterone (pg/ml) | 18.7 | 8.7 |
| Baseline cortisol (μg/dl)[c] | 0.08 | 0.04 |
| Baseline estradiol (pg/ml)[d] | 1.1 | 0.4 |
| Baseline testosterone (pg/ml) | 76.3 | 21.0 |

[a]Cared for any offspring 1 hr or more on the day of sampling, before the study.
[b]Subject reported having slept well or very well the night before the study.
[c]$n = 42$.
[d]$n = 39$.

## RESULTS

Table 1 summarizes the demographic, socioeconomic, and baseline hormonal characteristics of the study fathers. All men in the sample were married and had been so for an average of 5.4 years. A majority of the fathers (74%) had multiple children, and the men had been parents for 4.4 years, on average. Slightly under half (48%) of all fathers reported playing with their offspring everyday.

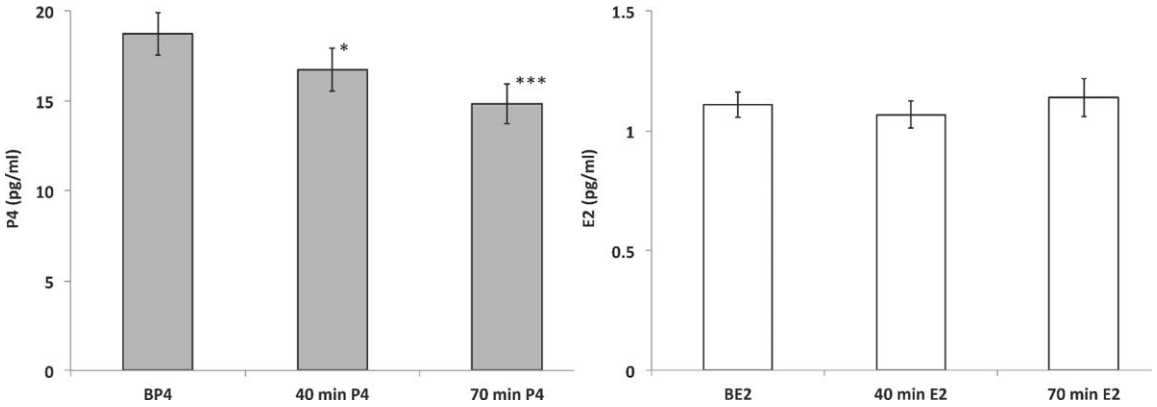


Fig. 1. Change in progesterone (P4) and estradiol (E2) over the course of the study period. Paired t-test comparisons of baseline P4 ($n = 44$) and E2 ($n = 39$) to 40 min and 70 min values, respectively. All hormonal values are adjusted for sampling time and prior night's sleep duration. Baseline P4 (BP4) was significantly higher than 40 min P4 (*$P = 0.035$) and 70 min P4 (***$P = 0.0009$). Error bars indicate SEM.

We first tested for relationships between baseline P4 (BP4) and E2 (BE2) and sampling time, which negatively predicted BE2 [$\beta$ (95% CI): −0.19 (−0.41 to 0.02); $R^2$: 0.082; $P = 0.078$] but not BP4 ($P = 0.156$). Next, because sleep dynamics might influence male reproductive steroid levels (Andersen et al., 2005; Leproult and Van Cauter, 2011), particularly among fathers (Gettler et al., 2012a), we tested whether men's sleep duration and wake time related to BP4 and BE2. While wake time was not significant for either hormone (both $P > 0.3$), subjects who slept longer the night before the study had lower BE2 [$\beta$:−0.08 (−0.15 to −0.02); $R^2$: 0.156; $P = 0.013$] and BP4 [$\beta$:−1.87 (−3.27 to −0.47); $R^2$: 0.148; $P = 0.010$]. We adjusted for sampling time and sleep duration in the subsequent analyses.

We then evaluated whether E2 and P4 were positively correlated to CORT (P4) and T (E2 and P4). BE2 was not significantly correlated to baseline T ($r = 0.03$; $P > 0.8$) while BP4 was significantly positively associated with baseline CORT ($r = 0.55$; $P = 0.0002$) and marginally correlated to T ($r = 0.26$; $P = 0.093$).

Consistent with our hypotheses, E2 did not significantly shift over the course of the interaction period (both $P > 0.4$; Fig. 1). BP4 was significantly higher than P4 40 min later ($P = 0.035$) and 70 min later ($P = 0.0009$; Fig. 1).

We then tested for between-father variability in E2 change ($n = 39$) during the study period based on fathers' characteristics, controlling for the duration of the father-child interaction as a continuous variable. Men's number of children ($P > 0.3$), fathers' routine involvement in play ($P > 0.1$), and men being father to an infant ($P > 0.1$) did not predict change in E2 between baseline and 40 min. Similarly, having an infant and playing frequently (both $P > 0.6$) did not influence E2 shifts between baseline and 70 min (%E70). Men with more offspring had a greater decrease in %E70 [$\beta$: −14.58 (−27.36 to −1.81); $R^2$: 0.153; $P = 0.026$]. We ran a similar model treating total interaction time as a series of dummy variables (see Methods). The effect size ($\beta$:−14.74) and significance ($P = 0.025$) were essentially unchanged.

Assessing similar models for P4 ($n = 44$), controlling for the duration of the interaction, we found that men's number of children ($P > 0.7$) and fathers' routine involvement in play ($P > 0.1$) did not predict percentage change in P4 between baseline and 40 min (%P40). However, men who were fathers of infants had a significantly greater decline in %P40 [$\beta$: −24.81 (−48.92 to −0.69); $R^2$: 0.123; $P = 0.044$] compared with fathers of older offspring. When we adjusted the model for total interaction time as a series of dummy variables, the effect size ($\beta$:−26.52) increased marginally, while significance was comparable ($P = 0.043$). After controlling for change in CORT, which reduced the sample size by two subjects ($n = 42$), the relationship became marginally significant [$\beta$: −25.64 (−51.71 to 0.44); $R^2$: 0.117; $P = 0.054$]. Paternal characteristics did not significantly predict change in P4 between baseline and 70 min (all $P > 0.3$).

We hypothesized that P4 exposure (P4 area under the curve; P4 AUC) during the study period would relate positively to fathers' post-intervention mood ratings. In a bivariate ANOVA ($n = 44$), P4 AUC was marginally related to higher paternal mood [model: $F_{(2,43)} = 2.96$; $R^2$: 0.126; $P = 0.063$]. Men who reported feeling very happy/relaxed after the interaction had higher P4 AUC than men reporting feeling mildly happy/relaxed ($P = 0.031$) and subjects reporting being neither upset/happy ($P = 0.048$). To account for the possibility that men with higher mood ratings might have simply experienced longer durations between saliva samples, contributing to higher P4 AUC, we controlled for the time between the baseline and second sample ($P = 0.088$) and baseline and third sample ($P = 0.135$). In this model, the relationship between paternal mood and P4 AUC was significant ($P = 0.020$).

Using ANCOVA ($n = 42$), controlling for CORT AUC ($P = 0.0004$) (Wirth and Schultheiss, 2006; Wirth et al., 2007) and time between baseline and saliva samples two and three (both $P > 0.3$), we found that men who reported feeling very happy/relaxed after the father-child interaction had greater P4 AUC (model: $F_{(5,41)} = 5.14$; $R^2$: 0.417; $P = 0.001$; Fig. 2) compared with men who reported being neither upset/happy ($P = 0.034$) or mildly happy/relaxed ($P = 0.034$). We adjusted the model for total interaction time ($P = 0.120$), rendering the relationship between P4 AUC and paternal mood non-significant ($P = 0.173$). Modeling total interaction time using dummy variables led to

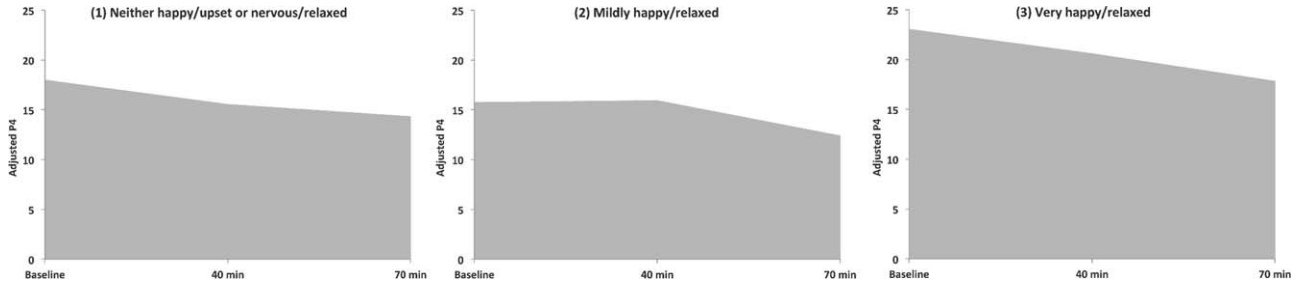


Fig. 2. Progesterone area under the curve (P4 AUC) by fathers' post-interaction mood ratings. All hormonal values are adjusted for sampling time, prior night's sleep duration, and CORT. See Results for full model details. Contrasts from ANCOVA, controlling for CORT: group 1 ($n = 22$) to 3 ($n = 11$), $P = 0.034$; group 2 ($n = 9$) to 3, $P = 0.034$.

virtually identical, non-significant results for paternal mood ($P = 0.173$), and men who interacted for 15–20 min trended towards lower P4 AUC ($P = 0.088$) compared with men who played with their toddlers for the full 30 min. In a separate bivariate model (ordered logistic regression), fathers who interacted with their toddlers for longer durations were more likely to report higher mood ratings [odds ratio (95% CI): 1.14 (1.02 to 1.28); $P = 0.024$].

Finally, as a visual inspection of Figure 2 indicates, very happy fathers tended to have higher BP4 (model: $F_{(2,43)} = 2.66$; $R^2$: 0.115; $P = 0.082$) than other subjects. Though the effect sizes ($F$, $R^2$) and significance are modestly lower than the comparable model for P4 AUC (above), one could make the argument that these results indicate a BP4-anticipatory effect on post-interaction mood.

## DISCUSSION

Here we present the first study to explore the potential responsiveness of E2 and P4 to human father-offspring interaction. Data on the roles of these hormones in human male psychobiology are generally limited; specifically, only a single study to date has examined paternal E2 (Berg and Wynne-Edwards, 2001), with no prior research on P4 in human fathers, to our knowledge. In terms of steroidogenesis, E2 is downstream of T, whereas P4 is a precursor to the production of both CORT and T. Given our previous findings that CORT declined and T remained stable during this father-child interaction protocol (Gettler et al., 2011a), we predicted that E2 would be unchanged and P4 would decrease over the course of the study period, with both hypotheses being supported. There is also growing evidence that P4 may reduce anxiety and is positively related to motivation for social affiliation (Wirth, 2011). We documented results consistent with this role of P4, as fathers who reported feeling happy/relaxed following the interaction had greater P4 exposure during the study period, on average, which persisted after controlling for CORT (Wirth and Schultheiss, 2006; Wirth et al., 2007). Finally we found that paternal characteristics predicted between-father variability in hormonal change, as fathers of infants showed a greater 40 min decline in P4 compared with fathers of older children and men with multiple children experienced a larger decrease in E2 over the full study period (~70 min).

In light of the comparatively large output of CORT (relative to P4) in males, and their dual production by the adrenals, including a significant positive correlation between baseline P4 and CORT in the present study, it is not surprising that the hormones showed similar patterns of acute decline in our study (Gettler et al., 2011a; Wirth et al., 2007). There are a few primary possible explanations for the observed parallel between P4 and CORT. In general, short-term changes in P4 may simply be secondary to the hormone's role as a precursor to CORT synthesis. Both hormones follow a broadly similar circadian rhythm, declining over the course of daytime, waking hours (Gröschl et al., 2003; Kage et al., 1982; Konishi et al., 2012). Thus, their mirrored patterns of decrease here could merely reflect commonalities in circadian biology. An additional, related possibility is that concurrent release of P4 and CORT may be functionally linked to the negative feedback loop regulating the activity of the hypothalamic-pituitary-adrenal axis (Wirth et al., 2007). That said, our finding that fathers of infants (mean: −21.9%) had a larger percentage decline in P4 (40 min) compared with fathers of older children (mean: −0.5%), which persisted after controlling for CORT, is suggestive of P4 regulation independent of both CORT production and circadian physiology.

Alternatively, the declines in P4 and CORT could be a direct result of father-child play stimulating a neurophysiological cascade that leads to CORT/P4 downregulation, independent of circadian biology. For example, we previously argued that reduced adrenal steroid production following father-child play could reflect the actions of oxytocin (Gettler et al., 2011a), which has been shown to rise during short-term father-child interactions (Feldman et al., 2010). Oxytocin reduces adrenal CORT output as well as the pituitary's release of CORT's stimulating peptide hormone, adrenocorticotropin (Heinrichs et al., 2003; Legros et al., 1984), processes that would likely downregulate the CORT's steroid precursors, such as P4.

An additional, contrary possibility is that P4 actually declined *less* during the father-child interaction than it would have under normal, non-stimulated conditions. Under this scenario, father-child play would mitigate, but not eliminate, the circadian physiology underpinning the hormone's typical diurnal decrease (Brown et al., 2009). This might help explain the positive relationship we observed between P4 and paternal mood, in spite of P4 declining during the study. Notably, our study lacked a control condition, in which the same subjects' hormones would have been observed during a comparable time frame, minus the stimulation of father-child interaction. This framework would have allowed us to more definitively parse out relationships between declines in P4, father-child play, and circadian physiology. Future studies in this area could expand on the present findings and

comparable CORT results elsewhere (Storey et al., 2011) through research designs oriented towards these distinctions.

Consistent with the function of P4 as an anxiety reducer or mood enhancer (Wirth, 2011), we found a positive relationship between P4 exposure during the study period and men's ratings of their mood following the interaction. These results remained significant after controlling for men's CORT, suggesting that associations between paternal mood and P4 are independent of generalized adrenal activation. The positive relationship between P4 and fathers' post-interaction mood ratings might reflect a P4-mediated dampening of anxiety (Wirth, 2011). Lending credence to this possibility, recent qualitative data from Cebu suggest that mothers and fathers engage in affectionate and playful behavior with their children as a specific way to ameliorate day-to-day stress (I. Bas, J. Avila, S. Agustin, and J. Borja, conference proceedings). The association between paternal mood and P4 became non-significant after adjustment for the total time fathers interacted with their toddlers. Total interaction time was itself related to paternal mood and (marginally) to P4 during the study period. These results suggest complex relationships between paternal P4, length of father-child interaction bouts, and paternal mood. For example, longer father-child play might contribute to elevated P4, which could then facilitate higher mood reports. Alternatively, fathers who are initially happier/more relaxed might play longer and more positively, which could elicit sustained P4 release. Anticipatory, elevated baseline P4 before parent-child interaction, as we observed here, could also contribute to this nexus by influencing play duration, mood, or both. These issues of causality and potential mutual reinforcement merit future consideration. In total, we suggest that our findings are consistent with a generalized correlation between P4 and enhanced mood (Wirth, 2011), rather than a psychobiological pathway restricted to father-child interaction. However, subsequent research could be designed to explore whether there are fathering-specific or parenting-specific (e.g. common to mother-child and father-child interactions) P4-mood relationships versus the likelihood that such socioendocrine patterns are simply common to positive, affiliative interactions in humans.

Finally, our exploratory findings that P4 and E2 responses varied based upon measures of paternal status (P4: father to an infant; E2: number of children), if replicated in other similar studies, could indicate that grouping men with different levels of experience and exposures obscures underlying heterogeneity in hormonal responsiveness. This would be consistent with prior results suggesting that men's paternal experience and caregiving history alter their hormonal responsivity (Delahunty et al., 2007; Gettler et al., 2011a; Storey et al., 2011) and basal hormone production (Gettler et al., 2011b; Jasienska et al., 2012). Recent research has established connections between variation in men's testosterone after father-child interaction and fathers' later brain activity in response to offspring stimuli (Kuo et al., 2012). This provides a fruitful framework for thinking about the ways in which inter-individual variability in hormonal patterning may impact men's parenting. Future studies with larger samples sizes will be better equipped to evaluate the connections between potential experiential (e.g. number of children) and situational (e.g. having an infant-aged offspring) programming effects on paternal E2 or P4 and their implications for the nature of father-child interactions.

Our study has limitations that warrant discussion. The lack of a control condition, as discussed above, constrains our ability to draw definitive conclusions regarding the causes of P4 patterning over the study period. In addition, it is possible that an unmeasured third variable (e.g. degree of father-child physical contact) could elicit greater P4 exposure and higher mood ratings, leading to positive correlations between P4 and mood. We did not record our father-child interactions nor were we able to code them for specific behavioral patterns in real time. These methods have proved fruitful in a recent study on father-child play and paternal physiology, providing an exemplar for future research (Feldman et al., 2010). In addition, our study was not specifically designed to assess paternal mood, thus the question asked of fathers following the interaction combined affective dimensions that are often measured separately [e.g. emotional valence (happy) versus arousal (relaxed)] (Bradley and Lang, 2000). This combined question format prevents us from specifically determining whether "happier" fathers or "more relaxed" fathers had higher P4 exposure. We also did not consider change in mood over the study period, thus it is plausible that men who were happier/more relaxed at baseline had higher BP4 and/or greater P4 exposure, rather than P4 causing enhanced mood. Our P4-mood analyses are somewhat limited by small sample sizes in the three mood categories. That we detected significant relationships in spite of this limitation suggests the study was adequately powered, but these relationships ought to be tested in future studies with larger sample sizes. In addition, recent research on a female sample suggests that there may be inter-individual variability in diffusion of P4 to saliva, leading to inconsistent correlations between salivary and circulating, serum P4 across subjects (Konishi et al., 2012). While we cannot specifically speak to potential P4 variability related to this physiological issue in our male subjects, we can think of no *a priori* reason that between-individual differences in "secretory mechanics" would account for relationships between P4 and paternal mood nor should this affect within-individual statistical comparisons of P4 change over the study period. Still, the issue merits consideration in studies implementing salivary P4 methodology (Konishi et al., 2012).

Lastly, we analyzed males' salivary E2 using a commercially available kit designed and validated for a normative range of female salivary E2 (Salimetrics LLC). The mean E2 values (Fig. 1) for our male sample correspond closely to the value of the lowest calibrator (1.0 pg/ml), which suggests that our results may be subject to more imprecision than results from samples with higher average E2. That said, the lowest observed E2 value (0.5 pg/ml) in our study is substantially greater than the kit's lower limit of detection (0.1 pg/ml), and our quality control statistics meet accepted standards. Moreover, this type of measurement error is not likely to introduce bias, but may increase the likelihood of type II error due to reduced statistical power. In combination with the seminal work of Berg and Wynne-Edwards (2001), we hope that our present research on paternal E2 might stimulate further study in this area, including the development of a high sensitivity E2 assay oriented towards normative ranges of male endocrine function.

In sum, our results point to the need for studies of paternal physiology to consider fathers' characteristics, including number and age of offspring, which growing evidence suggests may affect the functioning of multiple physiological systems in fathers. We also report the first evidence indicating that P4 levels respond to child interaction among human fathers. However, our findings paint a complex picture of P4 regulation, with overall P4 exposure relating to emotional states, as predicted, despite probable linkages between production of CORT and P4. Based on our results, men exposed to comparatively greater P4 during father-child interaction appear to have more positive feelings about such experiences. If these men were shown to subsequently have greater participation in childcare, this would support a role for P4 in facilitating and maintaining father-child bonding and paternal involvement. These possibilities highlight the importance of additional work aimed at elucidating the role of P4 in the psychobiology of human parental investment and the specific physiological pathways underlying relationships between father-child interaction, paternal mood, and P4 production.

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## LITERATURE CITED

Adair LS, Popkin BM, Akin JS, Guilkey DK, Gultiano S, Borja J, Perez L, Kuzawa CW, McDade T, Hindin MJ. 2011. Cohort profile: The cebu longitudinal health and nutrition survey. Int J Epidemiol 40:619–625.

Alvergne A, Faurie C, Raymond M. 2009. Variation in testosterone levels and male reproductive effort: Insight from a polygynous human population. Horm Behav 56:491–497.

Andersen ML, Martins PJF, D'Almeida V, Bignotto M, Tufik S. 2005. Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. J Sleep Res 14:83–90.

Austin PC, Hoch JS. 2004. Estimating linear regression models in the presence of a censored independent variable. Stat Med 23:411–429.

Bagatell CJ, Dahl KD, Bremner WJ. 1994. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl 15:15–21.

Berg SJ, Wynne-Edwards KE. 2001. Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. Mayo Clin Proc 76:582–592.

Bradley MM, Lang PJ. 2000. Measuring emotion: Behavior, feeling, and physiology. In: Lane RD, Nadel L, editors. Cognitive neuroscience of emotion. New York, NY: Oxford University Press. p 242–276.

Brown SL, Fredrickson BL, Wirth MM, Poulin MJ, Meier EA, Heaphy ED, Cohen MD, Schultheis OC. 2009. Social closeness increases salivary progesterone in humans. Horm Behav 56:108–111.

Childs E, Van Dam NT, Wit H. 2010. Effects of acute progesterone administration upon responses to acute psychosocial stress in men. Exp Clin Psychopharmacol 18:78.

Christiansen K, Winkler E. 1992. Hormonal, anthropometrical, and behavioral correlates of physical aggression in Kung San men of Namibia. Aggressive Behav 18:271–280.

Cushing BS, Wynne-Edwards KE. 2006. Estrogen receptor-alpha distribution in male rodents is associated with social organization. J Comp Neurol 494:595–605.

Daitzman R, Zuckerman M. 1980. Disinhibitory sensation seeking, personality and gonadal hormones. Pers Indiv Differ 1:103–110.

Delahunty KM, McKay DW, Noseworthy DE, Storey AE. 2007. Prolactin responses to infant cues in men and women: Effects of parental experience and recent infant contact. Horm Behav 51:213–220.

Ellison PT. 2001. On fertile ground: A natural history of human reproduction. Cambridge, MA: Harvard University Press. p 346.

Eriksson CJP, von der Pahlen B, Sarkola T, Seppä K. 2003. Oestradiol and human male alcohol-related aggression. Alcohol Alcohol 38:589–596.

Feldman R, Gordon I, Schneiderman I, Weisman O, Zagoory-Sharon O. 2010. Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent–infant contact. Psychneuroendocrinology 35:1133–1141.

Fleming AS, Ruble D, Krieger H, Wong PY. 1997. Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. Horm Behav 31:145–158.

Fleming AS, Steiner M, Anderson V. 1987. Hormonal and attitudinal correlates of maternal behavior during the early postpartum period in first-time mothers. J Reprod Infant Psyc 5:193–205.

Genazzani A, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Nappi R, Luisi S, Palumbo M, Purdy R. 1998. Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J Clin Endocrinol Metab 83:2099–2103.

Gettler LT. 2010. Direct male care and hominin evolution: Why male-child interaction is more than a nice social idea. Am Anthropol 112:7–21.

Gettler LT, Agustin SS, McDade TW, Kuzawa CW. 2011a. Short-term changes in fathers' hormones during father-child play: Impacts of paternal attitudes and experience. Horm Behav 60:599–606.

Gettler LT, McDade TW, Feranil AB, Kuzawa CW. 2011b. Longitudinal evidence that fatherhood decreases testosterone in human males. Proc Natl Acad Sci USA 108:16194–16199.

Gettler LT, Agustin SS, McDade TW, Kuzawa CW. 2012a. Does cosleeping contribute to lower testosterone levels in fathers? Evidence from the Philippines. PLoS One 7:e41559.

Gettler LT, McDade TW, Feranil AB, Kuzawa CW. 2012b. Prolactin, fatherhood, and reproductive behavior in human males. Am J Phys Anthropol 148:362–370.

Gladue BA. 1991. Aggressive behavioral characteristics, hormones, and sexual orientation in men and women. Aggressive Behav 17:313–326.

Gray PB, Anderson KG. 2010. Fatherhood: Evolution and human paternal behavior. Cambridge, MA: Harvard University Press.

Gray PB, Campbell BC. 2009. Human male testosterone, pair bonding and fatherhood. In: Ellison PT, Gray PB, editors. Endocrinology of social relationships. Cambridge, MA: Harvard University Press. p 270–293.

Gray PB, Parkin JC, Samms-Vaughan ME. 2007. Hormonal correlates of human paternal interactions: A hospital-based investigation in urban Jamaica. Horm Behav 52:499–507.

Gröschl M, Rauh M, Dörr HG. 2003. Circadian rhythm of salivary cortisol, 17α-hydroxyprogesterone, and progesterone in healthy children. Clin Chem 49:1688–1691.

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 54:1389–1398.

Jasienska G, Jasienski M, Ellison PT. 2012. Testosterone levels correlate with the number of children in human males, but the direction of the relationship depends on paternal education. Evol Hum Behav 33: 665–671.

Kage A, Fenner A, Weber B, Schoneshofer M. 1982. Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. Klin Wochenschr 60:659–666.

Konishi S, Brindle E, Guyton A, O'Connor KA. 2012. Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values. Am J Phys Anthropol 149:231–241.

Kuo PX, Carp J, Light KC, Grewen KM. 2012. Neural responses to infants linked with behavioral interactions and testosterone in fathers. Biol Psychol 91:302–306.

Law Smith MJ, Deady DK, Moore FR, Jones BC, Cornwell RE, Stirrat M, Lawson JF, Feinberg DR, Perrett DI. 2011. Maternal tendencies in women are associated with estrogen levels and facial femininity. Horm Behav 61:12–16.

Legros JJ, Chiodera P, Geenen V, Smitz S, Frenckell RV. 1984. Dose-response relationship between plasma oxytocin and cortisol and adrenocorticotropin concentrations during oxytocin infusion in normal men. J Clin Endocrinol Metab 58:105–109.

Leproult R, Van Cauter E. 2011. Effect of 1 week of sleep restriction on testosterone levels in young healthy men. JAMA 305:2173–2174.

Liening SH, Stanton SJ, Saini EK, Schultheiss OC. 2010. 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 99:8–16.

McCarthy MM, Arnold AP. 2011. Reframing sexual differentiation of the brain. Nat Neurosci 14:677–683.

Muller MN, Marlowe FW, Bugumba R, Ellison PT. 2009. Testosterone and paternal care in East African foragers and pastoralists. Proc R Soc B 276:347–354.

Numan M, Insel TR. 2003. The neurobiology of parental behavior. New York: Springer. p 418.
Nunes S, Fite JE, Patera KJ, French JA. 2001. Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*). Horm Behav 39:70–82.
O'Connor DB, Archer J, Wu FCW. 2004. Effects of testosterone on mood, aggression, and sexual behavior in young men: A double-blind, placebo-controlled, cross-over study. J Clin Endocrinol Metab 89:2837–2845.
Oettel M, Mukhopadhyay AK. 2004. Progesterone: The forgotten hormone in men? Aging Male 7:236–257.
Schneider JS, Stone MK, Wynne-Edwards KE, Horton TH, Lydon J, O'Malley B, Levine JE. 2003. Progesterone receptors mediate male aggression toward infants. Proc Natl Acad Sci USA 100:2951–2956.
Schum JE, Wynne-Edwards KE. 2005. Estradiol and progesterone in paternal and non-paternal hamsters (*Phodopus*) becoming fathers: Conflict with hypothesized roles. Horm Behav 47:410–418.
Söderpalm AHV, Lindsey S, Purdy RH, Hauger R, de Wit H. 2004. Administration of progesterone produces mild sedative-like effects in men and women. Psychoneuroendocrinology 29:339–354.
Storey AE, Noseworthy DE, Delahunty KM, Halfyard SJ, McKay DW. 2011. The effects of social context on the hormonal and behavioral responsiveness of human fathers. Horm Behav 60:353–361.
Storey AE, Walsh CJ, Quinton RL, Wynne-Edwards KE. 2000. Hormonal correlates of paternal responsiveness in new and expectant fathers. Evol Human Behav 21:79–95.
Trainor BC, Bird IM, Alday NA, Schlinger BA, Marler CA. 2003. Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. Neuroendocrinology 78:36–44.
Trainor BC, Marler CA. 2002. Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. Proc R Soc B 269:823–829.
van Anders SM, Goldey KL, Kuo PX. 2011. The Steroid/Peptide theory of social bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. Psychoneuroendocrinology 36:1265–1275.
Wallen K. 2005. Hormonal influences on sexually differentiated behavior in nonhuman primates. Front Neuroendocrinol 26:7–26.
Wingfield JC, Hegner RE, Ball GF, Duffy AM. 1990. The 'challenge hypothesis': Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136:829–846.
Wirth MM, Meier EA, Fredrickson BL, Schultheiss OC. 2007. Relationship between salivary cortisol and progesterone levels in humans. Biol Psychol 74:104–107.
Wirth MM. 2011. Beyond the HPA axis: Progesterone derived neuroactive steroids in human stress and emotion. Front Neuroendocrinol 2:1–14.
Wirth MM, Schultheiss OC. 2006. Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. Horm Behav 50:786–795.
Ziegler TE, Jacoris S, Snowdon CT. 2004. Sexual communication between breeding male and female cotton-top tamarins (*Saguinus oedipus*), and its relationship to infant care. Am J Primatol 64:57–69.