11-2018

Fatherhood and Psychobiology in the Philippines: Perspectives on Joint Profiles and Longitudinal Changes of Fathers' Estradiol and Testosterone

Lee T. Gettler

Patty X. Kuo

Sonny Agustin Bechayda

Published in *American Journal of Human Biology* 30:6 (November/December 2018), e23150;
doi: 10.1002/ajhb.23150

Submitted December 5, 2017; revised April 19, 2018; accepted May 29, 2018.

Supporting Information – Additional supporting information can be found following the references.

Fatherhood and Psychobiology in the Philippines: Perspectives on Joint Profiles and Longitudinal Changes of Fathers' Estradiol and Testosterone

Lee T. Gettler,[1,2,3,4] Patty X. Kuo,[1,3] and Sonny Agustin Bechayda[5]

1. Department of Anthropology, University of Notre Dame, Notre Dame, Indiana, USA
2. The Eck Institute of Global Health, University of Notre Dame, Notre Dame, Indiana, USA
3. William J. Shaw Center for Children and Families, University of Notre Dame, South Bend, Indiana, USA
4. The Eck Institute of Global Health, University of Notre Dame, Notre Dame, Indiana, USA
5. USC Office of Population Studies Foundation and Department of Anthropology, Sociology, and History, University of San Carlos, Cebu City, Philippines

Corresponding author – Lee Gettler, Department of Anthropology, University of Notre Dame, 244 Corbett Family Hall, Notre Dame, IN 46556 USA, email lee.t.gettler.1@nd.edu

ORCID Lee T. Gettler https://orcid.org/0000-0001-7325-8852

Abstract

Objectives: Research on the psychobiology of partnering and fathering has focused on testosterone (T), oxytocin, and prolactin (PRL) as mechanisms that potentially mediate life history trade-offs related to those roles. Less is known about other hormones that might be responsive to life history transitions and implicated in fathering, such as estradiol (E2). We examined how E2 changed during the transition to marriage and fatherhood, its correlation with fathers' caregiving, and its joint within-individual production with other hormones (T, PRL). *Methods:* Data were collected from a total of 913 Filipino men (aged 25.9 years ± 0.3 SD at follow-up) enrolled in a longitudinal cohort study. Morning saliva

samples collected at baseline (2005) and follow-up (2009) were assayed for T and E2 (n = 329), dried blood spots from baseline were assayed for PRL. Fathers reported on caregiving in 2009. *Results:* When compared with men who remained single non-fathers over the study period, men who became married residential fathers experienced larger declines in E2. This effect was non-significant when we controlled for longitudinal changes in T. E2 was not significantly related to fathers' caregiving, controlling for T. In cross-sectional analyses for PRL, T, and E2, married residential fathers exhibited within-individual profiles of reduced T and elevated PRL, whereas single non-fathers exhibited the opposite profile of elevated T and reduced PRL. *Conclusions:* Our findings point to the need for future research to consider the mutually regulatory dynamics and/or combinatorial implications of multiple physiological axes acting within individuals to underpin life history trade-offs and behavioral strategies.

1. Introduction

Life history theory-based perspectives on the physiological mechanisms that help mediate trade-offs between mating/competition and invested partnering and parenting tend to focus on the hormones testosterone (T) and, to a lesser extent, prolactin (PRL) and oxytocin (Gettler, 2014, 2016; Gray, McHale, & Carré, 2017; Roney & Gettler, 2015; Storey & Ziegler, 2016; van Anders, Goldey, & Kuo, 2011). Recent results suggest ways in which hormonal differences based on partnering and parenting may contribute to parents' physical and mental health (Gettler & Oka, 2016; Gettler, Sarma, Gengo, Oka, & McKenna, 2017; Saxbe et al., 2017), and paternal psychobiology influences fathers' investments in indirect care and sensitive, nurturing forms of direct care, which contribute to positive cognitive, social, and physical outcomes for children (Sarkadi, Kristiansson, Oberklaid, & Bremberg, 2008).

To expand the richness of our understanding of these biosocial phenomena from evolutionary, psychobiological, and applied perspectives, there is a need to increase the breadth of physiological signals included in such studies, and to begin to move toward analytical perspectives and research designs that enable us to test how various hormones act together or in opposition to one another to produce divergent behavioral and health outcomes (Bos, 2017; Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013; Gettler, 2014, 2016; Storey & Ziegler, 2016; van Anders et al., 2011). Indeed, less is known about other hormonal signals that might be responsive to life history transitions and implicated in expressions of fathering, such as estradiol (E2). E2 plays a negative feedback role within the hypothalamic-pituitary-gonadal axis (i.e., contributing to reduced T production), promotes adipose tissue accumulation in some areas on the body, is converted from T by adipose tissue (aromatization), and also has immunomodulatory functions. Thus, E2 is a hormone that is potentially relevant to life history trade-offs between costly forms of reproductive effort and survival/maintenance and also to questions regarding fatherhood, marriage, and men's health (Bribiescas, 2005; Gettler et al., 2017; Gettler, McDade, Feranil, Agustin, & Kuzawa, 2014; Raven, de Jong, Kaufman, & de Ronde, 2006).

Based on evidence from animal models of paternal biology, E2 potentially contributes to fathering, although the direction of the effects appears to vary, even within relatively closely related taxa. In at least 1 rodent species (*Phodopus californicus*) elevated E2 in the brain promotes paternal care, although peripheral levels remain low or even undetectable

(Trainor, Bird, Alday, Schlinger, & Marler, 2003; Trainor & Marler, 2002). In contrast, experimental paradigms with prairie voles (*Microtus ochrogaster*) and comparative perspectives involving multiple rodent species (i.e., in *Microtus* and *Phodopus*) indicate that elevated estrogenic activity in key brain areas interferes with paternal care of pups and other prosocial behaviors (Cushing, Perry, Musatov, Ogawa, & Papademetriou, 2008). Among New World monkeys, experienced cotton-top tamarin (*Saguinus oedipus*) fathers exhibit a rise in E2 in the months before young are born, which may be part of a suite of physiological changes that help prepare males for paternal involvement (Ziegler, Washabaugh, & Snowdon, 2004). Meanwhile, among black tufted-ear marmosets (*Callithrix kuhlii*) lower E2 coincides with heightened direct care (Nunes, Fite, & French, 2000). Comparatively, E2 remains understudied in research on human male social neuroendocrine function.

A study of Canadian men showed that expectant and new fathers' E2 was higher compared with non-fathers, preliminarily suggestive of direct upregulation of fathers' E2 in the peri-partum period (Berg & Wynne-Edwards, 2001). Contrasting with those results, a recent longitudinal study of pregnant women and their male partners found that fathers' E2 decreased across the pre-partum period and that fathers with larger E2 declines from the early to late pre-partum were more involved with household duties and care of infants after the birth (Edelstein et al., 2015; Edelstein et al., 2017). These findings closely align with prior research showing longitudinal declines in men's T when they transition to fatherhood and marriage in some settings (Edelstein et al., 2015; Edelstein et al., 2017; Gettler, McDade, Agustin, Feranil, & Kuzawa, 2015; Gettler, McDade, Feranil, & Kuzawa, 2011; Holmboe et al., 2017; Mazur & Michalek, 1998). These declines in T would be predicted to limit the available pool of T for conversion to E2, leading to reduced levels of the latter.

1.1. Potential co-regulatory hormone dynamics in partnering and parenting

Because various hormones interact with one another physiologically, modulate common neural circuits, and/or are mutually dependent because of how they are synthesized in the body (e.g., E2 and T), hormones may have interactive, additive, or antagonistic effects on behavior. Yet, most studies of human paternal psychobiology focus on each hormone in isolation rather than attempting to model their joint profiles or mutual effects (van Anders et al., 2011). Thus, a main goal of this report was to investigate joint profiles of multiple hormones (T, E2, and PRL). Past single-hormone approaches indicate there is a rationale for testing whether they are jointly regulated within fathers, and, as we will briefly describe, these three hormones are plausibly interconnected physiologically.

PRL receptors are located on cells in the pituitary gland that produce luteinizing hormone, which is necessary to stimulate testicular production of T and E2. PRL receptors are likewise located on Leydig cells in the testes, which produce T (Rastrelli, Corona, & Maggi, 2015). In men, circulating E2 derives from both direct gonadal (testes) production, and more so from local, tissue-specific conversion of T to E2 via the enzyme aromatase (Nieschlag, Behre, & Nieschlag, 2012). Given these physiological conversion dynamics, T and E2 are often positively correlated (Gettler et al., 2014), and it is likely that fathers' E2 will mirror their T to some extent. However, because E2 contributes to the negative feedback loop that regulates the hypothalamic-pituitary-gonadal axis and there is between-individual variation

in other aspects of its production (e.g., aromatase enzyme levels), its production and potential effects on male behavior could likewise be somewhat independent of T (Nieschlag et al., 2012; Raven et al., 2006). Finally, PRL release from the pituitary is primarily regulated by dopamine release from the hypothalamus. E2 attenuates dopamine's suppression of PRL production, leading to greater PRL with enhanced E2 (Ben-Jonathan & Hnasko, 2001).

Here, to help shed further light on the interplay between life history transitions, paternal caregiving, and men's hormones, including joint profiles thereof, we draw on both cross-sectional and longitudinal data from a sample of men enrolled in a large birth cohort study in Cebu, the Philippines. In this article, we address 4 main research questions. Our prior work from this study has shown that men's T declines over a 4.5-year follow-up period when they become newly married, new fathers, particularly if fathers reside with their children (Gettler et al., 2011; Gettler et al., 2015). Building from this prior work, our core analyses first tested for changes in E2 for men who were single non-fathers at baseline (aged 21.5 years ± 0.3 SD) and had either remained single non-fathers or experienced life history transitions to marriage and/or fatherhood (aged 25.9 years ± 0.3 SD). We hypothesized that men becoming newly married residential fathers would experience larger declines in E2 than men remaining single non-fathers. Second, we also tested whether fathers with lower E2 were more involved with childcare (Edelstein et al., 2017).

With the addition of E2 to this dataset, our study is one of the few that has assessed multiple hormones in men of varying life history statuses. This study provides the opportunity to model whether these hormones are jointly regulated within individuals based on partnering and parenting. Thus, using cross-sectional data from the baseline survey, we tested whether married residential fathers had distinct T, PRL, and E2 profiles compared with married and single non-fathers as well as married non-residential fathers. Finally, using longitudinal data from single non-fathers at baseline, we also tested whether single non-fathers who became married residential fathers by follow-up exhibited joint, within-individual changes in T and E2 that differentiated them from other men.

2. Methods

2.1. Study population

The participants in this study are enrolled in the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based study of infants (birth cohort) and their mothers that began in 1983–1984 in Cebu City, Philippines (Adair et al., 2011). The birth cohort subjects were interviewed in 2005 and 2009. This research was conducted under conditions of informed consent from the Institutional Review Boards of the University of North Carolina at Chapel Hill and Northwestern University.

2.2. Sociodemographics

During in-home visits, CLHNS team members collected socioeconomic, demographic, and behavioral data during interviews conducted in the local language (Cebuano). We classified men who identified themselves as being legally married or cohabitating as married (Gettler et al., 2011). In the 2005 CLHNS data collection, men reported their household composition, including the presence of their children, but did not report whether they had

children who were not residing with them. We used data from the 2009 survey to identify men who were non-residential fathers in 2005, which we have described previously in Gettler et al. (2015). In 2009, men reported whether they were fathers and their biological relatedness to their children. We defined men as fathers if they reported having biological children (Gettler et al., 2011, 2015). Of the 451 men who reported being fathers in 2009, 99% were biological fathers to at least 1 child (i.e., 1% were solely step- or adoptive-fathers).

2.3. Paternal care

In 2009, fathers reported their routine involvement in direct care in response to the question, "How much time do you usually spend providing physical care to your children on a daily basis?" We grouped men according to the following categories: low (less than an hour), moderate (1-3 h), and high (3+ h).

2.4. Salivary T and E2

During both surveys, participants collected a waking saliva sample in the morning (AM) and reported the time of collection. Mean AM sampling times were 6:48 AM ± 2:03 in 2005 and 6:55 AM ± 1:54 in 2009. These samples were shipped on dry ice to Northwestern University, where they were stored at −80°C. T and E2 levels were assayed using enzyme immunoassay protocols developed for use with saliva samples (Salimetrics, Carlsbad, California; Kit Numbers: T, 1-2402; E2, 1-3702). T was analyzed for all men who participated in the 2005 and/or 2009 surveys. Men's AM E2 was analyzed for a random sub-sample of men in the study because of financial constraints, as the laboratory costs were funded by a small internal grant to LTG during his graduate career. A small percentage (~2%) of the total saliva samples analyzed for E2 had at least one value that fell below the assay's level of detection. We ascribed these undetectable samples a value of 0.1 pg/mL, which is the lower limit of assay sensitivity. Married residential fathers are a key subgroup for our analyses below. When compared with men in other life history status categories, they were not more likely to have undetectable E2 (see Supporting Information).

For T, the interassay coefficients of variation were 13.7% and 11.5% for high and low kit-based control samples, respectively, in 2005 samples and 7.8% and 17.9% for high and low control samples, respectively, in 2009 samples. For E2, the samples for both surveys were run concurrently; the interassay CVs were 5.7% for the high and 3.8% for the low control samples, respectively. Four values were eliminated from these analyses on the basis of T or E2 values that were 4+ SD above the mean, indicating potential blood contamination.

2.5. Dried blood spot PRL

Single drops of men's blood were applied to Whatman protein cards for dried blood spot (DBS) analysis in 2005 only. A sub-sample of 308 men was later analyzed for PRL (see Supporting Information and Gettler, McDade, Feranil, & Kuzawa, 2012). We modified a serum-based assay (Diagnostic Systems Laboratories no. 10-4500) for use with DBS based on an existing validated protocol (Gettler et al., 2012). The inter-assay coefficients of variation for PRL were 10.8% and 24.9% for high and low control samples, respectively. Two individuals were excluded from our analyses based on PRL values 4+ SDs above the mean.

2.6. Anthropometrics and health-related covariates

Triceps skinfold thicknesses (mm) were measured using standard anthropometric techniques. Self-reported psychosocial stress in the month preceding sampling was quantified via a modified version of the 10-item Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983). We calculated men's sleep times (hours) using their self-reported typical wake and bed times. For longitudinal analyses, we calculated change scores between baseline and follow-up. We included triceps skinfold, sleep time, and PSS as covariates (see Supporting Information Methods for further information).

2.7. Statistical analyses

We conducted statistical analyses using both Stata 14.0 (Stata Corporation) and SPSS 24.0 (IBM Corporation). Hormone values were statistically adjusted for their sampling times prior to all other analyses. We used time-adjusted E2 and T values to calculate absolute change in the hormones between baseline (2005) and follow-up (2009) (Gettler et al., 2015). We present descriptive statistics for the sample in Table 1. Also primarily for descriptive purposes, we tested for differences between baseline and follow-up E2 using a Student's paired *t* test for the overall sample and a repeated measures (RMs) ANOVA to test for changes in E2 based on life history shifts. We also report associations between hormones using Pearson's correlation coefficients (*r*). Because the demographic patterns for men's marital and fatherhood status differed between 2005 and 2009, our analyses below vary slightly between the 2005 cross-sectional analyses and the 2005–2009 within-individual change analyses (see Supporting Information).

Using OLS linear regression and focusing on men who were single non-fathers at baseline, we predicted longitudinal within-individual changes in E2 between baseline and follow-up based on men's marital and fatherhood status at follow-up. In the base model, we included changes in men's triceps skinfold thickness, sleep time, and PSS as covariates. In a second model, we added changes in men's T as a covariate. We then used OLS regression to predict fathers' E2 at follow-up from their routine involvement in childcare. We included men's triceps skinfold thickness, sleep time, and PSS as covariates. In a second model, we added men's T as a covariate.

In order to identify joint hormonal profiles in single non-fathers, married non-fathers, married residential fathers, and married non-residential fathers in 2005, we ran a 4 (Life history status) × 3 (Hormone: T, E2, PRL) linear mixed model. The hormone × life history status interaction was modeled to assess whether hormonal profiles differed based on marital and fatherhood status. We conducted linear mixed models with full information maximum likelihood estimation to address missing data because the total sample sizes of participants eligible for these analyses for T ($n = 905$), PRL ($n = 302$), and E2 ($n = 315$) varied based on subsampling for PRL and E2.

Hormones were modeled as a repeated effect using an identity covariance matrix to account for within-subject covariation in T, E2, and PRL. A random effect of intercept using a variance components matrix was included to model between-participant individual differences. Missing data were accounted for using full information maximum likelihood.

Model fit was determined using Schwarz's Bayesian Information Criterion (BIC). BIC differences > 10 are considered strong evidence that the lower-BIC model fits best (Raftery, 1995). The model including a random intercept (BIC = 4302.99) did not have a difference larger than 10 from the model without a random intercept (BIC = 4295.81). Because of the large differences in values between T, E2, and PRL, hormone values as well as change scores for T and E2 were standardized prior to running our joint profile and longitudinal change models.

To identify joint changes in T and E2 in men experiencing life history transitions between 2005 and 2009, we ran a 4 (Life history status in 2009) × 2 (Hormone 2005–2009 change: T or E2) linear mixed model. The random effect of intercept was included to model individual differences between participants and was modeled using a variance components matrix. Again, model fit was determined using Schwarz's BIC, and removing the random intercept did not produce a BIC difference > 10. The BIC value for our final model was 2468.23. A two-way interaction between life history status and hormone change was included to test whether changes within T and E2 differed between single non-fathers, married non-fathers, married residential fathers, and single non-residential fathers. We used post hoc Fisher's least significant difference tests for pairwise comparisons.

Table 1. Descriptive statistics

Demographic characteristics	Mean	*SD*	*n*
Age 2005 (years)	21.5	0.3	913
Married/cohabitating 2005 (% y)	20.0	…	913
Father 2005 (% y)	14.8	…	913
Married/cohabitating 2009 (% y)	51.3	…	785
Father 2009 (% y)	49.5	…	785
Number of biological children	1.6	0.8	381
Education (highest grade)	10.7	3.3	785
Biomarker values			
AM estradiol 2005 (pg/mL)	2.2	0.9	315
AM estradiol 2009 (pg/mL)	2.0	0.7	321
AM testosterone 2005 (pg/mL)	190.9	75.8	905
AM testosterone 2009 (pg/mL)	163.5	62.9	785
PRL 2005 (ng/mL)	11.6	4.1	302
Paternal care[a,b]			
Low involvement in routine care (% y)	25.8	…	155
Moderate involvement in routine care (% y)	43.2	…	155
High involvement in routine care (% y)	31.0	…	155

Values are from follow-up (2009) unless otherwise noted.

a. See Methods for category definitions.

b. Father's caregiving data restricted to men who also had 2009 E2 values.

3. Results

For the full sample, men's E2 was higher at baseline than at follow-up (Student's paired *t* test: $t[313] = 4.65$, $P < .001$). In the RM ANOVA focused on men who were single non-fathers at baseline, men's E2 was also significantly lower at follow-up than baseline ($F[1245] = 12.03$, $P < .01$; Figure 1), but parenting/partnering status did not significantly moderate the overall effect of time, $P = .18$.

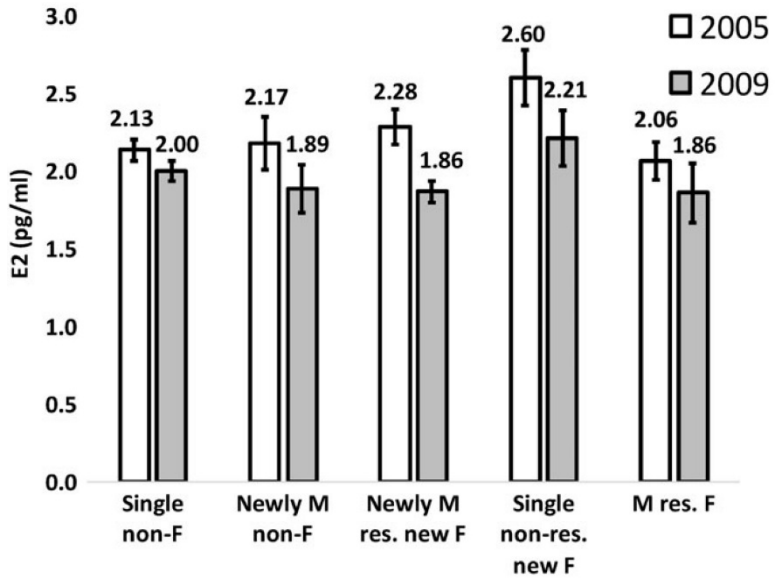

Figure 1. Men's baseline (2005) and follow-up (2009) salivary estradiol (E2) values, stratified according to life history status at baseline and follow-up. M = married; F = fathers; res. = residential. E2 from 2005 and 2009 for men who were single non-fathers at baseline and who remained single non-fathers at follow-up ($n = 136$), became newly married non-fathers ($n = 23$), became newly married residential new fathers ($n = 76$), became newly married non-residential new fathers ($n = 13$). For visual and descriptive purposes, we include data for men who were married residential fathers at both time points ($n = 27$), but they were not included in our analyses. See the results for model details. Error bars represent SE.

Across the entire sample, men with higher E2 at baseline also had higher E2 at follow-up ($r = 0.22$, $P < .001$). Men with greater E2 at baseline had lower PRL ($r = -0.20$, $P = .03$), while baseline T and PRL were not significantly associated ($P > 0.6$). Participants with higher T at baseline also had higher baseline E2 ($r = 0.14$, $P = .01$) and at follow-up ($r = 0.36$, $P < .001$). Within-individual changes in E2 and T across the study period were also positively correlated ($r = 0.21$, $P < .001$).

3.1. Assessing changes in E2 in fathers and non-fathers

Our OLS regression results showed that men who transitioned from being single non-fathers to being married residential fathers experienced larger declines in E2 compared with men who remained single non-fathers at both time points ($P < .05$; Table 2; Figure 2). Men who transitioned to being married non-fathers, married non-residential fathers, or single non-residential fathers across the study period did not significantly differ from the comparison group (P's > .2; Table 2; Figure 2). The difference between single non-fathers and men who became newly married residential fathers was no longer significant ($P > .1$) when we controlled for change in T ($P < .01$; Table 2).

Table 2. Predicting men's longitudinal change (Δ) in salivary E2 based on life history status between baseline and follow-up

	Model 1			Model 2		
	b	*SE*	*P*	*b*	*SE*	*P*
Men's marital and parenting status[a]						
Single non-F to married non-F	–0.12	(0.21)	.58	–0.10	(0.21)	.63
Single non-F to married residential F	–0.29	(0.14)	.03	–0.22	(0.14)	.10
Single non-F to single non-residential F	–0.31	(0.28)	.26	–0.29	(0.27)	.28
Covariates						
Δ triceps skinfold thickness	0.01	(0.01)	.19	0.02	(0.01)	.15
Δ sleep time	–0.01	(0.03)	.72	–0.01	(0.02)	.64
Δ PSS	0.02	(0.01)	.13	0.02	(0.01)	.13
Δ testosterone				0.002	(0.001)	.006
Model R^2		0.04			0.07	

Life history transitions reflect changes between 2005 and 2009. $n = 248$. F = fathers. PSS = perceived stress scale.

a. Comparison group: single non-F in 2005 and 2009 ($n = 136$). Single non-F to married non-F ($n = 23$). Single non-F to married residential F ($n = 76$). Single non-F to single non-residential F ($n = 13$).

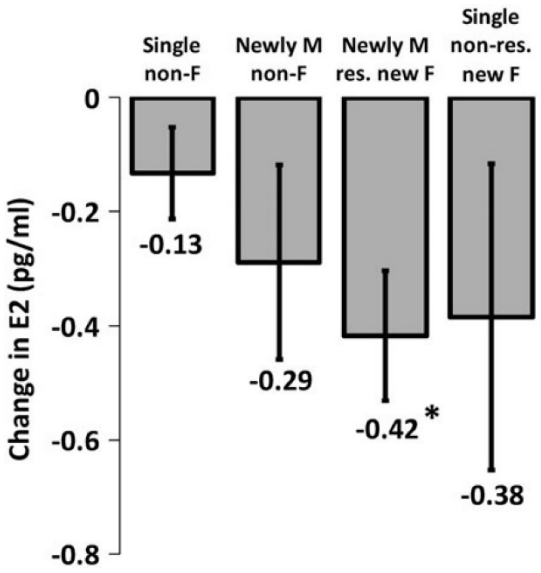

Figure 2. Change in E2 among men who were single non-fathers at baseline and stratified according to life history status at follow-up. M = married; F = fathers; res. = residential. E2 declined significantly more among newly married residential new fathers compared with men who remained single non-fathers (*$P < .05$). See Table 2 for full model details. Error bars represent SE.

3.2. Predicting E2 based on fathers' caregiving

Using OLS regression, we then tested whether fathers' caregiving predicted E2 at follow-up and whether those associations were independent of T, in light of our prior findings (Gettler et al., 2011; Gettler et al., 2015). Controlling for covariates, we found that fathers who engaged in moderate (1–3 h) daily childcare had significantly lower E2 than men who did little or no (0–1 h) daily care (b – 0.31, SE = 0.14, $P = .03$, model $R^2 = 0.07$; $n = 155$). Fathers who reported the highest daily care (3+ h) also tended to have lower E2 than the comparison group, though the difference was not statistically significant ($P = .10$). When we added T to the model, the effect sizes diminished and there were no longer significant E2 differences based on fathers' daily childcare (P's > .08).

3.3. Examining joint profiles of T, E2, and PRL in fathers and non-fathers

To test whether married residential fathers exhibited joint hormonal profiles that could distinguish them from single non-fathers, married non-fathers, and married non-residential fathers using the cross-sectional baseline data for T, E2, and PRL, we then ran a linear mixed model and interpreted the hormone × life history status interaction. Total sleep time, stress, and triceps skinfold thickness were included as covariates. Triceps skinfold thickness was a significant covariate ($F[1, 643.81] = 4.89$, $P < .05$). The interaction effect of hormone × father status was significant ($F[6, 1048.05] = 4.14$, $P < .001$). Post hoc pairwise comparisons revealed that married residential fathers had significantly lower T (mean $z = -0.42$, SE =

0.09) than single non-fathers (mean z = 0.07, SE = 0.04, P < .001) and married non-fathers (mean z = 0.005, SE = 0.14, P < .05). Married residential fathers had significantly higher PRL (mean z = 0.19, SE = 0.11) than single non-fathers (mean z = −0.10, SE = 0.07, P < .05). There were no significant differences in E2 between any of the groups (P's > .1). Among single non-fathers, standardized T levels were significantly higher than standardized PRL levels (P < .05) whereas among married residential fathers standardized T levels were significantly lower than standardized PRL levels (P < .001). There were no significant differences between standardized T, E2, or PRL levels within married non-fathers or non-residential married fathers. See Figure 3A-C for a plot of the hormone profiles.

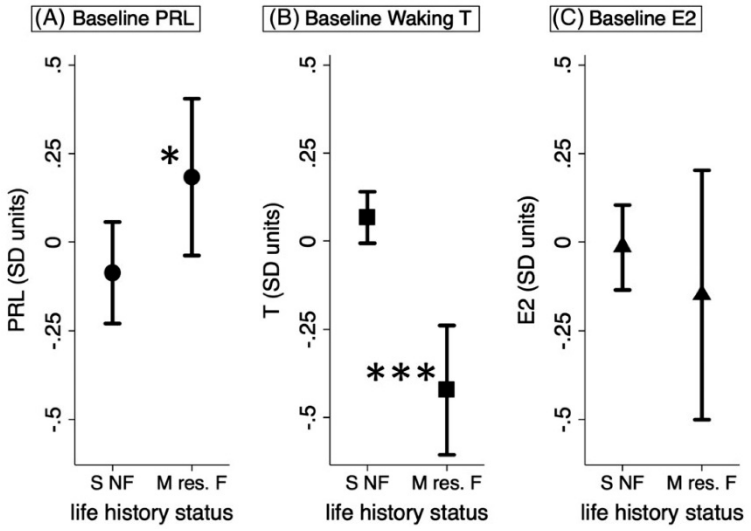

Figure 3. A-C, Standardized values of men's baseline PRL, T, and E: based on whether they were single non-fathers (S NF) or married residential fathers (M res. F). See the results for full model details. Figures produced using predictive margins following statistical models. Married residential fathers had lower T (*** P < .001) and higher PRL (* P < .05) than single non-fathers. The groups did not significantly differ for E2. Error bars represent 95% CI.

3.4. Evaluating joint changes in T and E2 among fathers and non-fathers

Results from the linear mixed model predicting joint changes in T and E2 with changes in triceps skinfold thickness, stress, and sleep as covariates revealed that the 2-way interaction between hormone change and life history status was not significant (P > .7). There was a significant main effect of father status (F[3, 582.56] = 4.20, P < .01). Post hoc pairwise comparisons revealed significant differences in overall T and E2 changes between single non-fathers (mean z = 0.09, SE = 0.05) and married residential fathers (mean z = –0.21, SE = 0.07, P < .001). Examination of the raw mean change scores revealed that married residential fathers exhibited greater declines in overall T and E2 (mean T change = –52.24, mean E2 change = –0.42) than single non-fathers (mean T change = –21.67, mean E2 change = –0.13).

4. Discussion

Our study is among the first as well as largest to examine longitudinal changes in men's E2 across life history transitions and to test for joint profiles of men's hormones related to life history status. These are significant steps toward understanding the complexity of the physiological axes and signals that have interactive, additive, or antagonistic effects in underpinning life history trade-offs and in shaping dynamics of men's health, such as could be related to mental health, body composition, and chronic disease risk (Gettler, 2014; Gettler et al., 2017; Saxbe et al., 2017; van Anders et al., 2011). Our results specifically suggest that changes in E2 as men transition to fatherhood may be largely driven by concomitant reductions in T production.

4.1. E2, life history transitions, and paternal care

When examining changes in E2, we found that E2 declined significantly in men who became newly married residential new fathers over the 4.5-year follow up period, compared with men remaining single non-fathers. In contrast, changes in E2 did not significantly differ for men who were single non-fathers at baseline and went through other life history transitions, relative to the comparison group. We also found some modest evidence for associations between lower E2 and fathers' greater participation in daily childcare. Both of these results for paternal psychobiology parallel recent work by Edelstein et al. (2015, 2017). In their study of U.S. men, they also found that E2 declines significantly across the transition to parenthood. Moreover, fathers with larger decreases in E2 across the pre-partum were more involved with infant care and household tasks in the first year post-partum (Edelstein et al., 2015, 2017). When compared with our single paternal care variable, their thorough assessment of divisions of household labor and infant care, reported from mothers and fathers, could potentially provide insights on familial dynamics that are differentially or independently related to T or E2. We suggest more theorizing is potentially necessary in this area but also that additional analytical/methodological considerations are imperative for effective modeling of E2 in such studies.

In our analyses, single non-fathers and newly married residential new fathers no longer differed significantly for changes in E2 after we accounted for change in T. Similarly, the modest negative relationship we found between fathers' caregiving and E2 was likewise accounted for by T. Because T also declines in men becoming married fathers at this site and is lower in men engaging in more childcare, these results suggest that levels of T account for much of the observed patterns for E2 (Gettler et al., 2011). Specifically, it is plausible that E2 levels may have decreased due to the lower levels of T available for conversion to E2. In the prior relevant work on human paternal physiology (Berg & Wynne-Edwards, 2001; Edelstein et al., 2015, 2017), concomitant changes in T were not included in models for E2. Thus, it is unclear whether these physiological dynamics might vary across populations. Furthermore, one difference between our study and prior studies is our focus on men's E2 changes during the transition to marriage and parenthood across a longer timeframe (4.5 years), compared with changes across the peri-partum period among expectant fathers (Berg & Wynne-Edwards, 2001; Edelstein et al., 2017). These potential physiological

variations and methodological issues will be important points of emphasis for future studies in this area.

In addition, there is increasing health-oriented research on the biosocial pathways through which men's adiposity increases as they become partnered and fathers in settings like the United States. Reduced T among partnered fathers is one likely physiological pathway through which this comes to fruition (Gettler et al., 2017). Adipose tissue also converts T to E2. Thus, in the long-term, fathers who experience lower T and heightened adiposity could end up with profiles of relatively elevated E2. Because men with higher E2 and lower T are also likewise more likely to store excess energy as fat in certain depots, this could have chronic health implications that merit further exploration (Gettler et al., 2014). Given that peripheral measurements of male E2, particularly in saliva, may be limited in terms of modeling behavioral effects (i.e., divergence between central and peripheral levels; also see Limitations in section 4.3), incorporating E2 into perspectives on marriage and fatherhood as components of men's long-term health, including body composition, may be a particularly fruitful area for future research. This is likely to be especially beneficial if studies employ saliva sampling approaches that enable detailed assessment of individuals' hormone production across the day (e.g., area under the curve; Gettler et al., 2014) and use more sensitive assay techniques (Gao, Stalder, & Kirschbaum, 2015).

4.2. Joint hormone profiles

Psychobiological models recognize the functional intersections of multiple neuroendocrine signals in promoting complex social behaviors, such as the expression of reactive aggression, the competitive pursuit of status and dominance, and the formation of social bonds (Mehta & Josephs, 2010; van Anders et al., 2011). Despite relevant frameworks (van Anders et al., 2011), few studies of human paternal psychobiology have modeled whether fathers' hormones are mutually regulated within individuals. Here, we found evidence for mutual regulation of PRL and T but not E2 among married residential fathers (higher PRL, lower T) and single non-fathers (lower PRL, higher T). Along these lines, scholars studying fathers' biology across vertebrate taxa recognize complementary roles for elevated PRL and reduced T in shaping paternal investment (Gettler, 2014; Storey & Ziegler, 2016). Consistent with this idea, our cross-sectional models focusing on men during young adulthood indicate the potential for joint regulation of T and PRL, in opposing directions, among married residential fathers and single non-fathers, which may emerge through two pathways.

First, PRL and T may directly, mutually regulate one another. Men with health conditions causing elevated PRL (i.e., hyperprolactinemia) often have suppressed T, likely through PRL acting at the level of the brain or pituitary gland to ultimately attenuate the release of luteinizing hormone, which stimulates the production of T from the testes. However, under normal physiological circumstances (i.e., excluding conditions of hyperprolactinemia), PRL does not typically suppress men's T (Rastrelli et al., 2015). Because the levels of elevated PRL documented among human fathers generally do not approach the range of values associated with hyperprolactinemia (Gettler et al., 2012), it is unclear whether PRL-induced suppression of paternal T is likely to occur. A nonhuman primate analog hints that it is possible that this occurs among invested fathers, specifically. Among common marmosets (*C. jacchus*), a New World monkey species exhibiting intensive paternal care,

when fathers' PRL is experimentally suppressed, their T remains relatively elevated during the early post-partum, whereas it typically declines around that time (Ziegler, Prudom, Zahed, Parlow, & Wegner, 2009). Marmoset fathers with experimentally suppressed PRL were also less responsive to infant cues (Ziegler et al., 2004), which is indicative of the potential reproductive effort and parenting implications of reduced T and elevated PRL co-occurring within fathers under typical conditions. Moreover, PRL also plays an important metabolic role in facilitating weight gain in these marmoset fathers in preparation for the energetic demands of carrying their twins and triplets. The expectant fathers did not show this weight gain when their PRL was suppressed (and T was relatively elevated, which may attenuate adiposity accumulation); these results may be relevant to evolutionary-oriented energetic models of human fatherhood as well as contemporary fathers' health (Gettler, 2010; Gettler et al., 2017).

Second, rather than the two hormones directly regulating each other, their joint profiles may result from fathering experiences activating neural networks that have parallel regulatory effects on the hypothalamic neurons that ultimately downregulate T (via GnRH) and upregulate PRL (via dopamine). For example, in our prior work from Cebu, we have shown that fathers' T declines most steeply and that their PRL is most elevated during the first year of their infants' lives (Gettler et al., 2011, 2012). Thus, sensory signals from their infants and partners, interactions with them, and cognitive and emotional processes related to that intensive stage of fathering may have neural effects that influence functions of the hypothalamus, yielding joint profiles of reduced T and elevated PRL. In single-hormone approaches, both lower T and higher PRL have also been linked to greater paternal participation in caregiving and higher quality fathering behaviors (Bos, 2017; Gettler, 2014; Storey & Ziegler, 2016). In future studies involving these hormones and others, such as vasopressin, oxytocin, and cortisol, multi-hormone perspectives can provide critical insights on the ways in which some constellations of hormonal function have synergistic, additive effects on expressions of sensitive, nurturing fathering while others facilitate harsher practices (Bos, 2017).

4.3. Limitations and assay considerations for future research

Our study has limitations that are worthy of discussion. To assay men's salivary E2, we used a commercially available immunoassay kit that is optimized for a normative range of female salivary E2 (Salimetrics LLC; Gettler et al., 2014). Relatively few samples in our study (~2%) had undetectable values for E2 and married residential fathers (a key demographic sub-group for our analyses) were not more likely than other men to have undetectable E2 at baseline or follow-up. Still, the mean E2 values (Figure 1) for our male sample are in the lower range of the standard curve for this assay, near the lowest calibrator values of 1.0–2.0 pg/mL. These assay limitations do not introduce systematic bias, such as might increase the incidence of Type I errors, but do reduce the reliability of our E2 data and thereby limit our ability to detect significant relationships. Indeed, because data with reduced reliability should predispose our analyses to Type II error, three aspects of our results give us confidence that our E2 measures are capturing meaningful biological variation in this study. First, we found significant positive correlations between men's T and E2, as would be expected. Second, we showed that men's E2 changed across life history

transitions in ways that parallel our past longitudinal results for T (i.e., relatively larger declines among men becoming married residential fathers). Finally, bridging those two points, when we included change in T in the E2 models, it accounted for the patterns of change in E2 based on life history status.

In addition, prior research has shown that salivary and serum E2 are less strongly correlated among males compared with the associations in females. This past work indicates that salivary E2 might not serve as a particularly reliable proxy for circulating levels (among men), for example, compared with serum-salivary correlations for T (Shirtcliff et al., 2000). Consequently, research such as ours may be underpowered to detect E2's associations with behavioral patterns (Shirtcliff et al., 2000). We think it is useful to point out that there has been recent growth in the development of highly reliable and sensitive approaches to measuring salivary steroids, including E2, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays (e.g., Gao et al., 2015). For scholars with the expertise and access to employ these approaches, they may be a preferable option to the use of immunoassays, especially for males' E2. However, LC-MS/MS approaches require more costly equipment than typical immunoassays and may not be financially and logistically feasible (e.g., access to equipment; expertise) for many scholars, particularly for human biologists who conduct field-based research and must also budget for those time and resource costs. In total, human social neuroendocrine research would greatly benefit from the development of a high sensitivity immunoassay oriented toward normative ranges of male E2, and researchers interested in incorporating E2 into their research on fathering should also be attentive to current limitations of measuring E2 in men's saliva (Berg & Wynne-Edwards, 2001; Gettler et al., 2014; Shirtcliff et al., 2000).

5. Conclusion

In conclusion, we found that Filipino men transitioning from being single non-fathers to married residential fathers over a 4.5-year period experienced declines in E2 that were comparatively larger than men who remained single non-fathers over the same time span. These differences in E2 were accounted for by concomitant changes in T, likely reflecting T being upstream of E2 physiologically. Our results are complementary to recent findings from an intensive longitudinal study of expectant U.S. fathers (Edelstein et al., 2015, 2017); yet, methodological and empirical discrepancies between our study and other relevant work highlight the need for further research and theorizing on the psychobiological implications of E2 and its variation based on life history status and environmental factors, such as familial, ecological, and cultural contexts. Our cross-sectional findings that demonstrate joint, within-individual profiles for T and PRL among married residential fathers and single non-fathers, respectively, point to the need for future research to consider the mutually regulatory dynamics and/or combinatorial implications of multiple physiological axes acting within individuals to underpin life history trade-offs and behavioral strategies.

Acknowledgments – We extend our gratitude to the participants in the CLHNS for their multi-decade commitment to this study. Linda Adair, Judith Borja, Chris Kuzawa, and Thom McDade played central roles in designing and implementing components of the CLHNS study from which a portion of

these data and samples were obtained. Jeffrey Huang and Aaron Miller helped with lab work. We thank the Office of Population Studies Foundation, University of San Carlos, Cebu, Philippines, for their role in study design and data. This study was supported by the Wenner Gren Foundation (Gr. 7356; Gr. 8186), National Science Foundation (BCS-0542182; BCS-0962212), and Northwestern University. LTG was funded by a Wenner-Gren Foundation Hunt Postdoctoral Writing Fellowship during the writing of this article.

Conflict of Interest – The authors declare that they have no conflicts of interest with the contents of this article.

Author Contributions – All authors read and approved the final version of the article.
Conceptualized core aspects of the study design, secured funding for portions of the work, analyzed the data, and wrote the article: Gettler
Contributed to theoretical framing, analyzed the data, and wrote the article: Kuo
Collected data in the field, helped with framing of the article, and contributed to editing and writing of the article: Bechayda

References

Adair, L. S., Popkin, B. M., Akin, J. S., Guilkey, D. K., Gultiano, S., Borja, J., . . . Hindin, M. J. (2011). Cohort profile: The Cebu longitudinal health and nutrition survey. *International Journal of Epidemiology, 40*(3), 619–625. https://doi.org/10.1093/ije/dyq085

Ben-Jonathan, N., & Hnasko, R. (2001). Dopamine as a prolactin (PRL) inhibitor. *Endocrine Reviews, 22*(6), 724–763. https://doi.org/10.1210/edrv.22.6.0451

Berg, S. J., & Wynne-Edwards, K. E. (2001). Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. *Mayo Clinic Proceedings, 76*(6), 582–592.

Bos, P. A. (2017). The endocrinology of human caregiving and its intergenerational transmission. *Development and Psychopathology, 29*(3), 971–999. https://doi.org/10.1017/S0954579416000973

Bribiescas, R. G. (2005). Age-related differences in serum gonadotropin (FSH and LH), salivary testosterone, and 17-β estradiol levels among ache Amerindian males of Paraguay. *American Journal of Physical Anthropology, 127*(1), 114–121. https://doi.org/10.1002/ajpa.20079

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385–396. https://doi.org/10.2307/2136404

Cushing, B. S., Perry, A., Musatov, S., Ogawa, S., & Papademetriou, E. (2008). Estrogen receptors in the medial amygdala inhibit the expression of male prosocial behavior. *The Journal of Neuroscience, 28*(41), 10399–10403. https://doi.org/10.1523/jneurosci.1928-08.2008

Edelstein, R. S., Chopik, W. J., Saxbe, D. E., Wardecker, B. M., Moors, A. C., & LaBelle, O. P. (2017). Prospective and dyadic associations between expectant parents' prenatal hormone changes and postpartum parenting outcomes. *Developmental Psychobiology, 59*(1), 77–90. https://doi.org/10.1002/dev.21469

Edelstein, R. S., Wardecker, B. M., Chopik, W. J., Moors, A. C., Shipman, E. L., & Lin, N. J. (2015). Prenatal hormones in first-time expectant parents: Longitudinal changes and within-couple correlations. *American Journal of Human Biology, 27*(3), 317–325. https://doi.org/10.1002/ajhb.22670

Feldman, R., Gordon, I., Influs, M., Gutbir, T., & Ebstein, R. P. (2013). Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. *Neuropsychopharmacology, 38*(7), 1154–1162. https://doi.org/10.1038/npp.2013.22

Gao, W., Stalder, T., & Kirschbaum, C. (2015). Quantitative analysis of estradiol and six other steroid hormones in human saliva using a high throughput liquid chromatography-tandem mass spectrometry assay. *Talanta, 143*, 353–358. https://doi.org/10.1016/j.talanta.2015.05.004

Gettler, L. T. (2010). Direct male care and hominin evolution: Why male-child interaction is more than a nice social idea. *American Anthropologist, 112*(1), 7–21. https://doi.org/10.1111/j.1548-1433.2009.01193.x

Gettler, L. T. (2014). Applying socioendocrinology to evolutionary models: Fatherhood and physiology. *Evolutionary Anthropology: Issues, News, and Reviews, 23*(4), 146–160. https://doi.org/10.1002/evan.21412

Gettler, L. T. (2016). Becoming DADS: Considering the role of cultural context and developmental plasticity for paternal socioendocrinology. *Current Anthropology, 57*(S13), S38–S51. https://doi.org/10.1086/686149

Gettler, L. T., McDade, T. W., Agustin, S. S., Feranil, A. B., & Kuzawa, C. W. (2015). Longitudinal perspectives on fathers' residence status, time allocation, and testosterone in the Philippines. *Adaptive Human Behavior and Physiology, 1*(2), 124–149. https://doi.org/10.1007/s40750-014-0018-9

Gettler, L. T., McDade, T. W., Feranil, A. B., Agustin, S. S., & Kuzawa, C. W. (2014). Salivary estradiol and testosterone in Filipino men: Diurnal patterns and relationships with adiposity. *American Journal of Human Biology, 26*(3), 376–383. https://doi.org/10.1002/ajhb.22528

Gettler, L. T., McDade, T. W., Feranil, A. B., & Kuzawa, C. W. (2011). Longitudinal evidence that fatherhood decreases testosterone in human males. *Proceedings of the National Academy of Sciences of the United States of America, 108*(39), 16194–16199. https://doi.org/10.1073/pnas.1105403108

Gettler, L. T., McDade, T. W., Feranil, A. B., & Kuzawa, C. W. (2012). Prolactin, fatherhood, and reproductive behavior in human males. *American Journal of Physical Anthropology, 148*(3), 362–370. https://doi.org/10.1002/ajpa.22058

Gettler, L. T., & Oka, R. C. (2016). Are testosterone levels and depression risk linked based on partnering and parenting? Evidence from a large population-representative study of US men and women. *Social Science & Medicine, 163*, 157–167.

Gettler, L. T., Sarma, M. S., Gengo, R. G., Oka, R. C., & McKenna, J. J. (2017). Adiposity, CVD risk factors and testosterone variation by partnering status and residence with children in US men. *Evolution, Medicine, and Public Health, 2017*(1), 67–80. https://doi.org/10.1093/emph/eox005

Gray, P. B., McHale, T. S., & Carré, J. M. (2017). A review of human male field studies of hormones and behavioral reproductive effort. *Hormones and Behavior, 91*, 52–67. https://doi.org/10.1016/j.yhbeh.2016.07.004

Holmboe, S. A., Priskorn, L., Jørgensen, N., Skakkebaek, N. E., Linneberg, A., Juul, A., & Andersson, A.-M. (2017). Influence of marital status on testosterone levels—a ten-year follow-up of 1113 men. *Psychoneuroendocrinology, 80*(Supplement C), 155–161. https://doi.org/10.1016/j.psyneuen.2017.03.010

Mazur, A., & Michalek, J. (1998). Marriage, divorce, and male testosterone. *Social Forces, 77*(1), 315–330. https://doi.org/10.1093/sf/77.1.315

Mehta, P. H., & Josephs, R. A. (2010). Testosterone and cortisol jointly regulate dominance: Evidence for a dual-hormone hypothesis. *Hormones and Behavior, 58*(5), 898–906. https://doi.org/10.1016/j.yhbeh.2010.08.020

Nieschlag, E., Behre, H. M., & Nieschlag, S. (2012). *Testosterone: Action, Deficiency, Substitution*. Cambridge, UK: Cambridge University Press.

Nunes, S., Fite, J. E., & French, J. A. (2000). Variation in steroid hormones associated with infant care behaviour and experience in male marmosets (*Callithrix kuhlii*). *Animal Behaviour, 60*(6), 857–865. https://doi.org/10.1006/anbe.2000.1524

Raftery, A. E. (1995). Bayesian model selection in social research. *Sociological Methodology, 25*, 111–163. https://doi.org/10.2307/271063

Rastrelli, G., Corona, G., & Maggi, M. (2015). The role of prolactin in andrology: What is new? *Reviews in Endocrine and Metabolic Disorders, 16*(3), 233–248. https://doi.org/10.1007/s11154-015-9322-3

Raven, G., de Jong, F. H., Kaufman, J.-M., & de Ronde, W. (2006). In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. *The Journal of Clinical Endocrinology & Metabolism, 91*(9), 3324–3328. https://doi.org/10 .1210/jc.2006-0462

Roney, J. R., & Gettler, L. T. (2015). The role of testosterone in human romantic relationships. *Current Opinion in Psychology, 1*(Suppl C), 81–86. https:// doi.org/10.1016/j.copsyc.2014.11.003

Sarkadi, A., Kristiansson, R., Oberklaid, F., & Bremberg, S. (2008). Fathers' involvement and children's developmental outcomes: A systematic review of longitudinal studies. *Acta Paediatrica, 97*(2), 153–158. https://doi.org/10. 1111/j.1651-2227.2007.00572.x

Saxbe, D. E., Edelstein, R. S., Lyden, H. M., Wardecker, B. M., Chopik, W. J., & Moors, A. C. (2017). Fathers' decline in testosterone and synchrony with partner testosterone during pregnancy predicts greater postpartum relationship investment. *Hormones and Behavior, 90*, 39–47. https://doi .org/10.1016/j.yhbeh.2016.07.005

Shirtcliff, E. A., Granger, D. A., Schwartz, E. B., Curran, M. J., Booth, A., & Overman, W. H. (2000). Assessing estradiol in biobehavioral studies using saliva and blood spots: Simple radioimmunoassay protocols, reliability, and comparative validity. *Hormones and Behavior, 38*(2), 137–147. https://doi. org/10.1006/hbeh.2000.1614

Storey, A. E., & Ziegler, T. E. (2016). Primate paternal care: Interactions between biology and social experience. *Hormones and Behavior, 77*, 260–271. https://doi.org/10.1016/j.yhbeh.2015.07.024

Trainor, B. C., Bird, I. M., Alday, N. A., Schlinger, B. A., & Marler, C. A. (2003). Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. *Neuroendocrinology, 78*(1), 36–44.

Trainor, B. C., & Marler, C. A. (2002). Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*(1493), 823–829. https://doi.org/10.1098/rspb.2001.1954

van Anders, S. M., Goldey, K. L., & Kuo, P. X. (2011). The steroid/peptide theory of social bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. *Psychoneuroendocrinology, 36*(9), 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001

Ziegler, T. E., Prudom, S. L., Zahed, S. R., Parlow, A. F., & Wegner, F. (2009). Prolactin's mediative role in male parenting in parentally experienced marmosets (*Callithrix jacchus*). *Hormones and Behavior, 56*(4), 436–443. https://doi.org/10.1016/j.yhbeh.2009.07.012

Ziegler, T. E., Washabaugh, K. F., & Snowdon, C. T. (2004). Responsiveness of expectant male cottontop tamarins, *Saguinus oedipus*, tomate's pregnancy. *Hormones and Behavior, 45*(2), 84–92. https://doi .org/10.1016/j.yhbeh.2003.09.003

Supporting Information

Sampling, methods, and analytical considerations for salivary estradiol (E2)

To construct the subject pool for E2 analysis, we used Stata's "sample" command (Stata Corporation) to draw a random sample of 355 participants who had full data from both 2005 and 2009. Of these men, 331 had sufficient AM saliva from both time points for E2 analysis. The assay we used to measure male E2 is designed for a normative range of female E2 (Salimetrics LLC). Consequently, a small percentage (~2%; $n = 15$) of the total saliva samples analyzed for E2 had at least one E2 value that fell below the assay's level of detection. As in our prior research using this approach (Gettler et al., 2014), we ascribed these undetectable samples values of 0.1 pg/ml, which is the lower limit of assay sensitivity, according to the assay manufacturer (Salimetrics LLC). For similar reasons, we assayed only the men's AM saliva samples, which we predicted would have higher E2, on average, because of the diurnal curve of T and E2 (Gettler et al., 2014).

As shown in the Results (Table 2), men who transitioned from being single non-fathers at baseline to married residential fathers at follow-up experienced larger declines in E2 compared to men who remained single non-fathers across the study period. In Supplemental Table 1, we show the *n*'s and percentages of men in those longitudinal analyses with detectable E2 at both baseline and follow-up versus those who had at least one undetectable E2 value at either time point. Men who became married residential fathers were not more likely to have undetectable E2 ($p > .8$). In cross-sectional analyses restricted to baseline and follow-up, respectively, married residential fathers were similarly not more likely to have undetectable E2 at either time point (chi-square; both $p > 0.5$).

Supplemental Table 1. Percentages of men with detectable E2 at baseline and follow-up, among participants who were single non-fathers at baseline

Life history status at follow-up	Total *n*	*n* detectable E2 at both time points (%)	*n* undetectable E2 at either time point (%)
Single non-fathers	136	129 (95%)	7 (5%)
Married non-fathers	22	22 (96%)	1 (4%)
Married residential fathers	76	73 (96%)	3 (4%)
Single non-residential fathers	13	13 (100%)	0 (0%)

Chi-square, $p > .8$; total $n = 248$, matching the sample in Table 2

Sampling and methods for dried blood spot prolactin (PRL)

Single drops of men's blood were applied to Whatman protein cards for dried blood spot analysis in 2005 only. A sub-sample of 308 men was later analyzed for PRL (also see Gettler et al., 2012). For this sub-sample, we over-sampled men who reported being fathers in 2005

and drew a random sub-sample of men who were not fathers, yielding a total sub-sample of 308 men out of approximately 916 total participants. Similar to the description in the Methods for E2, it was necessary to analyze a sub-sample of the broader CLHNS study because of financial constraints on the project, which was part of Gettler's dissertation. In particular, because PRL is a rarely analyzed analyte in human biology research, the kits used in this study were comparatively, substantially more expensive (e.g., relative to those for many salivary steroid hormones). We assayed the samples using a commercially available immunoassay kit designed to measure PRL from serum (Diagnostic Systems Laboratories #10-4500). We modified this assay for use with dried blood spots based on a previously published, validated protocol for the same procedure (Gettler, McDade, Feranil, & Kuzawa, 2012). The inter-assay coefficients of variation for PRL were 10.8% and 24.9% for high and low control samples, respectively. The CLHNS did not collect blood samples in 2009, thus we only have PRL data for 2005 (Gettler et al., 2015). Two individuals were excluded from our analyses based on PRL values 4+ SDs above the mean.

Baseline (2005) versus longitudinal (2009) participant pools and life history status categories

For the 2005 cross-sectional analyses, we focused on the following categories: single non-fathers, married non-fathers, married residential fathers, and married non-residential fathers. There were very few men who were single non-residential fathers in 2005 and who had PRL ($n = 2$) or E2 ($n = 3$) data. In the 2005 to 2009 within-individual change models, which were restricted to single non-fathers at baseline, we focused on the following categories at follow-up: single non-fathers, married non-fathers, married residential fathers, and single non-residential fathers. There were few eligible men who became married non-residential fathers at follow-up and who had E2 data from baseline and follow-up ($n = 6$).

Additional information regarding covariates

We included triceps skinfolds as marker of energetic status because research suggests that men in some settings gain adiposity when they transition to marriage and fatherhood and E2 is related to elevated adiposity, including at this site (Gettler et al., 2014; Gettler et al., 2017). While less studied for E2, elevated psychosocial stress and reduced/poorer sleep are potentially linked to lower T and also commonly differ between parents and non-parents, thus we controlled for those factors (Leproult & Van Cauter, 2011; Sapolsky, Romero, & Munck, 2000).

References

Gettler, L. T., McDade, T. W., Feranil, A. B., Agustin, S. S., & Kuzawa, C. W. (2014). Salivary estradiol and testosterone in Filipino men: Diurnal patterns and relationships with adiposity. *American Journal of Human Biology, 26*(3), 376–383. doi:10.1002/ajhb.22528

Gettler, L. T., McDade, T. W., Feranil, A. B., & Kuzawa, C. W. (2012). Prolactin, fatherhood, and reproductive behavior in human males. *American Journal of Physical Anthropology, 148*(3), 362–370. doi:10.1002/ajpa.22058

Gettler, L. T., McDade, T. W., Agustin, S. S., Feranil, A. B., & Kuzawa, C. W. (2015). Longitudinal perspectives on fathers' residence status, time allocation, and testosterone in the Philippines. *Adaptive Human Behavior and Physiology, 1*(2), 124–149. doi:10.1007/s40750-014-0018-9

Gettler, L. T., Sarma, M. S., Gengo, R. G., Oka, R. C., & McKenna, J. J. (2017). Adiposity, CVD risk factors and testosterone variation by partnering status and residence with children in US men. *Evolution, Medicine, and Public Health, 2017*(1), 67–80. doi:10.1093/emph/eox005

Leproult, R., & Van Cauter, E. (2011). Effect of 1 week of sleep restriction on testosterone levels in young healthy men. *JAMA, 305*(21), 2173–2174.

Sapolsky, R. M., Romero, M. L., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev, 21*(1), 55–89.