Prolactin, Fatherhood, and Reproductive Behavior in Human Males

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ABSTRACT Although humans are considered unusual among mammals for the intensity of care that fathers often provide offspring, little is known about the hormonal architecture regulating human paternal investment. Prolactin has important reproductive functions in both female and male mammals and other taxa, making it a candidate regulator of human paternal behavior. Notably, prolactin is higher during periods of offspring care in some species, but it is unknown if this pattern occurs in human fathers. We draw on a sample of men (n = 289; age 21–23 at baseline) from Metropolitan Cebu City, Philippines to evaluate relationships between prolactin, assayed from dried blood spots, and components of reproductive behavior and relationship status. In this sample, fathers had higher prolactin than nonfathers (P = 0.006), and fathers of infants had borderline higher prolactin than fathers of older children (P = 0.054). Among single nonfathers at baseline (2005), baseline prolactin did not predict who transitioned to fatherhood by follow-up 4.5 years later. Among nonfathers, men with greater prolactin reported more lifetime sexual partners (P = 0.050) as well as more sexual activity in the month before sampling (P = 0.060). Our results suggest that fathers in Cebu have higher prolactin than nonfathers, with hormone levels highest among fathers of young infants. Although these findings are generally consistent with evidence from other species for pronurturing effects of prolactin, evidence for positive relationships between the hormone and measures of sexual behavior at Cebu point to likely complexities in the hormone's involvement in male reproductive strategy. Am J Phys Anthropol 148:362–370, 2012.

Mothers are primarily responsible for raising mammalian offspring, and the neuroendocrine pathways through which maternal investment is facilitated and maintained are well-documented (Numan and Insel, 2003; Broad et al., 2006). Perhaps because paternal care occurs in only ~5% of mammalian species, of which humans are one example (Kleiman, 1977; Kleiman and Malcolm, 1981; Clutton-Brock, 1991), much of the research on male reproductive behavioral physiology has focused on the neuroendocrine underpinnings of males' investments in mating effort. In particular, significant attention has been paid to the effects of testosterone (T), the principal male reproductive steroid. Multiple studies have shown that human fathers have lower T than nonfathers (Gray et al., 2006; Alvergne et al., 2009), especially among populations in which paternal care is common (Muller et al., 2009; Gettler et al., 2011b). This is consistent with the notion that promotion of mating-related activities is attenuated in the context of committed investment in child rearing. However, little is presently known about hormones that may increase with fatherhood to actively facilitate behavioral or emotional states related to successful childcare.

Prolactin (PRL) is a candidate hormone for such a role in human males. PRL is best known for its role in lactation and maternal nurturing behavior. In lactating females, suckling stimulates release of PRL, which is a principal regulator of milk production (Freeman et al., 2000). Rodent studies show that PRL acts in concert with steroid hormones to facilitate maternal behavior, such as pup retrieval or nest building (Voci and Carlson, 1973; Bridges et al., 1990; Bridges and Mann, 1994). Expression of PRL receptors is also upregulated in key brain areas related to social behavior during pregnancy and lactation (Pi and Grattan, 1999; Grattan et al., 2001), and gene knockout studies have documented the importance of PRL receptor expression to maternal caretaking behavior in nonhuman mammalian species (Lucas et al., 1998).

Although PRL is widely known for its role in mammalian maternal physiology and behavior, it evolved early in the vertebrate lineage, prior to the emergence of mammals (Forsyth and Wallis, 2002). During the course of vertebrate evolution PRL has been integrated into a wide range of physiological processes and is known to have over 300 biological functions across extant species, leading it to be described as the most versatile of all hormones

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(Bern and Nicoll, 1968; Freeman et al., 2000; Ben-Jonathan et al., 2008). Research on the role of PRL in taxa that last shared a common ancestor with mammals prior to the split of the mammalian lineage from early amniotes (e.g., fish, amphibians, reptiles) suggests that PRL served a range of ancestral functions. Although description of the many functions of PRL in these taxa are beyond the scope of this paper (for review, see Bern and Nicoll, 1968; De Vlaming, 1979), the hormone has been shown to have important osmoregulatory (fish and amphibians), somatotropic (reptiles, amphibians, fish) and metamorphic/developmental (amphibians) effects. There is also evidence that PRL helps regulate reproductive morphology and physiology (fish and amphibians) as well as mating behavior (amphibians) and paternal investment (fish) (Bern and Nicoll, 1968; De Vlaming, 1979). The array of physiological actions of PRL across taxa, including its numerous pleiotropic functions in mammals and birds (De Vlaming, 1979; Freeman et al., 2000), suggest that the hormone is easily exapted as novel physiological and behavioral functions arise.

Cross-species evidence indicates that PRL has independently evolved to promote paternal investment in three separate lineages (fish, birds, mammals) (Schradin and Anzenberger, 1999), highlighting the likelihood that it could have evolved to serve a similar function in the hominin lineage leading to modern *Homo sapiens* (Gettler, 2010; Gray and Anderson, 2010). Although male–female cooperation in caregiving is relatively rare in fish, uni-parental male care is common and might be facilitated by PRL (De Vlaming, 1979; Clutton-Brock, 1991). For example, studies have shown that PRL is necessary for normal expression of paternal care (Kindler et al., 1991) and exogenous PRL administration reduces males' courtship behaviors and facilitates male care of eggs (Blüm and Fiedler, 1965; Páll et al., 2004). Moreover, among seahorse species in which males incubate eggs on their bodies, PRL is necessary for maintenance of the brooding pouch and normal embryo development (Stölting and Wilson, 2007).

Because there are broad similarities between the ways in which both humans and birds practice cooperative breeding, representing convergent reproductive strategies (Ketterson and Nolan, 1994; Gray and Anderson, 2010), studies of bird physiology provide insights into the potential role of PRL in human fatherhood (Ziegler, 2000). Exogenous PRL has been shown to induce paternal care in male birds with breeding experience (Buntin et al., 1991), and there is also convincing evidence that an increase in PRL either precedes or coincides with the periods during which male birds directly care for young (e.g., during egg incubation) (Gratto-Trevor et al., 1990; Mauget et al., 1995; Lormee et al., 1999; Lormee et al., 2000) or contribute to provisioning (Ketterson et al., 1990; Wingfield and Goldsmith, 1990; Schoech et al., 1996). It has been suggested that for species in which males are absent from the mother or chicks for extended periods, such as is common in some pelagic birds, PRL remains elevated throughout the egg/chick dependency period to ensure continuity of paternal care (Lormee et al., 1999; Lormee et al., 2000). This work demonstrates the importance of PRL in male birds' parental investment while also emphasizing species-specific modulation of stimulation and maintenance of elevated PRL.

Fewer studies have investigated the role of PRL in mammalian male reproductive behavior, especially in relation to the possibility that PRL has been favored by selection to influence paternal care in the variety of mammalian lineages in which it has independently evolved (Clutton-Brock, 1991). For example, in multiple rodent species in which males help care for young, fathers' PRL is higher after the birth of their offspring (Gubernick and Nelson, 1989; Brown et al., 1995; Reburn and Wynne-Edwards, 1999). In a comparison of two closely related hamster species, this postpartum PRL increase was only observed in fathers of the species in which paternal investment is naturally expressed (Reburn and Wynne-Edwards, 1999). In striped mice, males can shift between being group living breeders that help with offspring care and roaming nonpaternal individuals throughout their reproductive careers. In this species, it was recently shown that PRL increased when males transitioned from roaming to breeding-caregiving (Schradin and Yuen, 2011). Similar upregulations of PRL have been documented in some nonhuman primate species. Among common marmosets, fathers were shown to experience a nearly twofold increase in PRL post-partum, which declined when caregiving diminished (Schradin and Anzenberger, 2004). Other studies found that marmoset males showed significantly higher PRL after carrying infants (Dixson and George, 1982; Mota et al., 2006). Multiple studies of cotton-top tamarins have also shown that experienced fathers have significantly higher PRL than first-time or less experienced fathers, suggesting that male tamarins incrementally upregulate PRL as they become increasingly established as fathers and caregivers (Ziegler et al., 1996, 2000).

Although these studies point to relationships between PRL and fathering behavior in various mammalian species, data from studies in which males' PRL production has been experimentally manipulated have yielded inconsistent effects on paternal behavior (Wynne-Edwards and Timonin, 2007). For instance, studies of several mammalian species have shown that administration of dopamine agonists, which suppress PRL production, failed to alter paternal care (Brooks et al., 2005; Almond et al., 2006). Conversely, a recent study found that marmoset paternal responsiveness was disrupted when fathers' PRL was experimentally altered outside of the normal physiological range (Ziegler et al., 2009).

Although a growing number of human studies have documented differences in T based on associations with relationship or fatherhood status (Gray et al., 2004; Gray et al., 2006; van Anders and Watson, 2006; Alvergne et al., 2009; Kuzawa et al., 2009; Muller et al., 2009), or that it declines with the onset of fatherhood (Gettler et al., 2011b), few comparable studies have evaluated the social or relationship predictors of PRL or the implications of the hormone for human fathers' participation in childcare. There is preliminary evidence suggesting that PRL may reduce males' mating effort, as older men with elevated PRL report reduced sexual desire and lower sexual activity in some studies (Weizman et al., 1983; Paick et al., 2006). Similarly, hyperprolactinemia is associated with lower sexual interest among men (Buvat, 2003), and experimental lowering of men's PRL has been shown to increase multiple measures of sexual drive (Kruger et al., 2003). Other results suggest a possible role of PRL in human paternal care, as Canadian fathers with higher basal PRL reported greater concern when hearing infant cries (Storey et al., 2000), and Israeli fathers with higher PRL showed greater time in exploratory play with their infants during observations (Gordon et al., 2010). However, other recent studies have found short-term declines

in PRL during father-child interaction (Gettler et al., 2011a; Storey et al., 2011), and the only previous study to evaluate PRL in relation to fatherhood status found that baseline PRL was comparable between Jamaican fathers and nonfathers (Gray et al., 2007). Thus, unlike the relatively well-supported relationship between PRL and paternal care in other taxa with biparental investment, it remains unclear whether PRL is elevated in human fathers or how normal variation in PRL relates to men's reproductive behaviors, such as sexual activity and parental caregiving.

To clarify the relationship between PRL, fatherhood status, and reproductive behaviors in human males, we use data and samples collected in 2005 and 2009 in a large sample of young men (n = 289: age 21–23 in 2005) living in Metropolitan Cebu City, Philippines. Drawing on these data, we test the following hypotheses: (1) men who were fathers at baseline (2005) will have higher PRL compared with men who were nonfathers, with PRL highest in fathers reporting greater involvement in childcare; (2) among nonfathers at baseline, men with higher PRL will be more likely to subsequently have become fathers and parental caregivers by follow-up (2009); and (3) PRL will be inversely related to mating effort, as indicated by men's number of lifetime sexual partners as well as their sexual activity in the month preceding PRL measurement.

MATERIALS AND METHODS

Study population

Data come from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based birth cohort study of infants born in 1983–1984 (Adair et al., 2011). The men studied here include a subsample (n = 289) ranging in age from 20.8 to 22.6 years at the time of data and sample collection at baseline (2005). Socioeconomic, demographic, health and general behavioral data were collected using questionnaire-based, in-home interviews administered by Cebuano-speaking interviewers (Adair et al., 2011). Self-reported psychosocial stress in the month preceding sampling was quantified via a modified version of the 10-item Perceived Stress Scale (PSS) (Cohen et al., 1983). Men self-reported their recent sexual activity at the baseline interview in response to the question "How often have you had sex in the last month?" using a 5-point scale ranging from "never" to "2 or more times per week." Men who responded "never" or "not applicable" were categorized as having no sexual activity in the last month. Men who reported having sex 1 time or more per week were grouped together. This research was conducted under conditions of informed consent with human subjects clearance from the Institutional Review Boards of the University of North Carolina, Chapel Hill, and Northwestern University.

Fatherhood and relationship status

Men were classified as "cohabitating partnered" if they identified themselves as married or in a cohabitating relationship, as "noncohabitating partnered" if they reported being in a romantic relationship that did not include marriage/cohabitation, and as single ("nonpartnered") if they did not report being in a romantic relationship. Fathers were defined as men who reported having one or more biological children. Among subjects with otherwise complete data for this analysis, 1 man at baseline and 3 men at follow-up reported having adopted, but no biological, children, and no men reported having only step-children. Because there were few fathers in noncohabitating relationships (n = 4) at baseline, these men were grouped with cohabitating fathers and described collectively as "partnered fathers." Using interview dates at baseline, children's ages at follow-up, and representative mean gestational lengths for this population (Adair et al., 2011), we were able to calculate the number of expectant fathers (n = 21), whose partners' were pregnant at the time of blood collection. All expectant fathers were partnered, with the exception of two subjects. At baseline, men were coded as being involved in childcare if they named themselves as one of the individuals who had primary responsibility for taking care of children in the household (Kuzawa et al., 2009). At follow-up, paternal caregiving was assessed via the question, "How much time do you usually spend providing physical care to your children on a daily basis?" with men grouping themselves by no contact/0 minutes, less than an hour, 1–3 hours, and 3+ hours (Gettler et al., 2011b).

Dried bloodspot collection and hormone measurement

DBS samples. In 2005, participants were asked to fast overnight for 12 h, and blood samples were collected in clinics the following morning using EDTA-coated tubes. Mean time of blood draw was 7:05 AM ± 0:36 (SD). Single drops of whole blood were immediately applied to Whatman protein cards for dried blood spot (DBS) analysis, which were allowed to dry at room temperature before being frozen until shipment on dry ice to Northwestern University where they were stored at −80°C until analysis.

DBS PRL. Assays for PRL were run at the Laboratory for Human Biology Research (LHBR) at Northwestern University using a commercially available kit designed to measure PRL from serum (Diagnostic Systems Laboratories # 10-4500). We modified this assay for use with DBS based on a previously published, validated protocol for the same procedure (Gray et al., 2007). The interassay coefficients of variation for PRL were 10.8% and 24.9% for high and low control samples, respectively.

Statistical analyses

All analyses were conducted using version 10 of Stata (Stata Corporation, College Station, TX). In all statistical models PRL variables were adjusted for usual wake time and time of blood collection. After calculating descriptive statistics, we used multiple linear regression to predict PRL from parenting/relationship status, controlling for covariates and confounders. We then used multiple linear regression to predict PRL from a series of variables related to fatherhood and multiple logistic regression to test whether PRL predicted who had become a first-time father by 2009. Applying ordered logistic regression, we predicted men's self-reported hours spent in direct physical childcare (2009) from baseline PRL among men who were nonfathers in 2005. Next, we predicted men's number of lifetime sexual partners from PRL, which required the use of negative binomial regression because of overdispersed count data. One subject was excluded for reporting an implausibly high number of lifetime sexual

TABLE 1. Sample characteristics[a]

	Total sample, $n = 289$	Median split of PRL		P value[b]
		Low PRL, $n = 145$	High PRL, $n = 144$	
Hormone variables				
PRL (ng/mL)	11.7 ± 4.1	8.7	14.8	0.0001
Time of sampling	7:05 ± 0:36	7:07	7:03	0.44
Usual wake time	7:22 ± 2:10	7:22	7:23	0.96
Demographic characteristics				
Age (years)	21.5 ± 0.3	21.5	21.5	0.46
Education (highest grade)	9.4 ± 3.2	9.6	9.3	0.48
Relationship and fatherhood status				
Single nonfather (%)	35.0%	38.6%	31.3%	0.19
Noncohabitating partnered nonfather (%)	27.3%	29.7%	25.0%	0.38
Cohabitating partnered nonfather (%)	9.0%	10.3%	7.6%	0.42
Single father (%)	0.7%	0.7%	0.7%	0.75
Noncohabitating partnered father[c] (%)	1.4%	1.4%	1.4%	0.99
Cohabitating partnered father[c] (%)	28.0%	20.7%	35.4%	0.005
Sexual activity				
Number of lifetime sexual partners	2.1 ± 3.2	2.2	2.1	0.79
Weekly sexual intercourse[d] (%)	24.2%	22.8%	25.7%	0.56
Fatherhood characteristics[e]				
Number of children	1.3 ± 0.5	1.2	1.4	0.10
Primary childcare provider (%)	39.8%	41.9%	38.5%	0.75
Father of an infant[f] (%)	53.0%	41.9%	59.6%	0.12

[a] Mean ± SD. All descriptive statistics are from baseline (2005).
[b] Unpaired t-test or chi-square across a median split of PRL. Low PRL < 11.4 (ng/mL) < High PRL.
[c] In statistical analyses, noncohabitating and cohabitating partnered fathers were combined.
[d] Frequency of sexual activity in the month prior to sampling.
[e] $n = 83$.
[f] Infant: offspring 1 year old or less.

partners. Finally, we used ordered logistic regression to predict men's sexual activity in the month prior to baseline sampling from PRL. All ordered logistic regression models met the parallel regression assumption based upon the Brant test. Two subjects were excluded because of PRL values 5+ SD above the mean of the sample. Statistical significance was set at $P < 0.05$ with relationships between $P > 0.05$ and $P < 0.10$ interpreted as a borderline statistical trend.

RESULTS

Descriptive statistics for the sample are provided in Table 1. In this study, on average, subjects were 21.5 years old and had completed slightly under a 10th grade education. Approximately a fourth (28%) of the men were cohabitating fathers at baseline (2005) whereas 35% were single nonfathers and 27% reported being nonfathers in noncohabitating romantic relationships. Subjects reported a mean of 2.1 lifetime sexual partners. A minority (40%) of fathers reported being one of the primary persons responsible for childcare at baseline and roughly half (53%) of fathers had an infant-aged (1-year-old or less) offspring.

We first predicted PRL from parenting status at baseline, showing that nonfathers ($n = 206$) had lower PRL (β −1.49, 95% CI −2.54, −0.44; $P = 0.006$; Fig. 1A) than fathers ($n = 83$). These results were essentially unchanged (β −1.48, 95% CI −2.52, −0.43; $P = 0.006$) after adjustment for psychosocial stress, the effects of which might have confounded relationships between PRL and fatherhood at age 21–23. We then stratified men according to both romantic relationship and parenthood status at baseline. Compared with partnered fathers, single nonfathers ($P = 0.012$) and noncohabitating partnered nonfathers ($P = 0.022$) had significantly lower PRL (Fig. 1B). The coefficients and significance were attenuated modestly after adjusting for psychosocial stress (Table 2).

Because cross-species evidence suggests that PRL may vary by paternal experience and the age of young, we then compared PRL of expectant fathers ($n = 21$) and fathers of older children ($n = 39$) to the PRL of fathers with infant-aged offspring ($n = 44$). Compared with fathers of infants, fathers of older children showed a trend towards lower PRL ($\beta = -1.68$, 95% CI −3.38, 0.03; $P = 0.054$; Fig. 2) and expectant fathers had lower PRL, but not significantly ($\beta = -1.71$, 95% CI −3.83, 0.42; $P = 0.114$; Fig. 2). The coefficients and significance decreased for both fathers of older children ($\beta = -1.59$, 95% CI −3.29, 0.11; $P = 0.067$) and expectant fathers ($\beta = -1.59$, 95% CI −3.71, 0.53; $P = 0.140$) after controlling for psychosocial stress.

When we limited our analysis to men who were already fathers at baseline ($n = 83$), testing whether parenthood-related variables predicted PRL, we found that fathers with multiple children had comparable PRL to fathers with one child ($P = 0.802$). There was also no significant difference between first-time fathers with infants and fathers of multiple children who had infants ($P = 0.967$). Additionally, fathers who reported more childcare at baseline did not have higher PRL than less involved fathers, contrary to our prediction ($P = 0.398$).

We next tested whether baseline PRL (before fatherhood) predicted which men became new parents by follow-up 4.5 years later and how intensively they were involved as caregivers if they became first-time fathers. Drawing on men who were nonfathers in 2005 ($n = 185$), we did not find that men with higher PRL were more likely to have become fathers by 2009 (OR 1.03, 95% CI 0.96, 1.11; $P = 0.369$). Among those men who became first-time fathers between baseline and follow-up

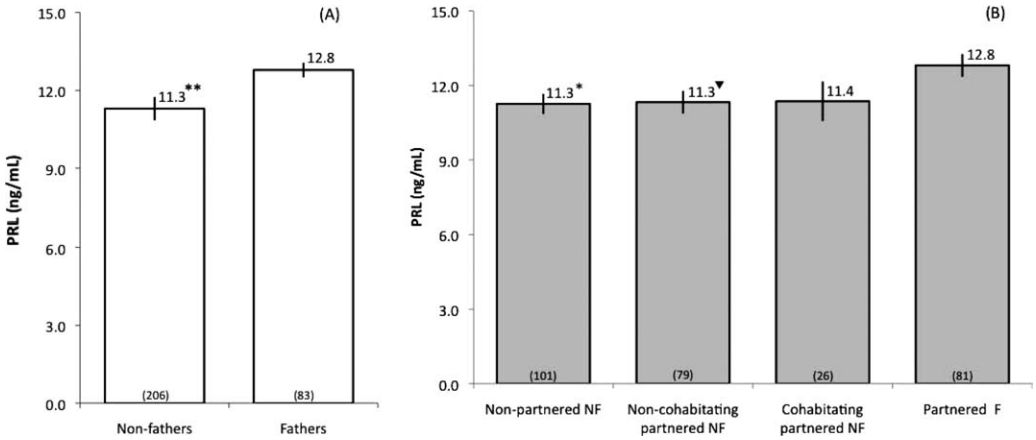

Fig. 1. A,B: PRL stratified according to whether men were fathers at baseline. Values were derived from regressing PRL on fatherhood status, with fathers as the comparison group, $^{**}P = 0.006$. **B**: PRL stratified according to men's relationship and fatherhood status at baseline. Values were derived from regressing PRL on relationship and fatherhood status, with partnered fathers as the comparison group, $^{*}P = 0.012$, $^{\blacktriangledown}P = 0.022$. NF, nonfather; F, father. Models for A and B control for time of blood collection and usual wake time. Data for single fathers ($n = 2$) not shown. Error bars indicate S.E.M.

TABLE 2. Multiple regression models predicting PRL[a] (n = 289)

	Model 1	*P*	Model 2	*P*
Relationship and fatherhood status[b]				
Single nonfathers ($n = 101$)	−1.55 ± 0.61	0.01	−1.52 ± 0.61	0.01
Noncohabitating partnered nonfathers ($n = 79$)	−1.48 ± 0.65	0.02	−1.49 ± 0.64	0.02
Cohabitating partnered nonfathers ($n = 26$)	−1.45 ± 0.92	0.12	−1.46 ± 0.92	0.11
Single fathers ($n = 2$)	−0.86 ± 2.91	0.77	−1.06 ± 2.91	0.72
Self-reported psychosocial stress (PSS)			0.06 ± 0.05	0.26
Model R^2	0.035		0.039	

[a] Values are $\beta \pm$ SE of PRL. PRL is adjusted for time of sample collection and usual wake time.
[b] Excluded comparison: partnered fathers ($n = 81$).

($n = 84$), baseline PRL did not predict how much direct care they provided to their children at follow-up (OR 1.02, 95% CI 0.92, 1.12; $P = 0.768$).

Finally, because various lines of human and cross-species evidence hint that PRL may reduce men's mating effort, we assessed whether men with higher PRL reported lower sexual activity. Contrary to this hypothesis, among men who were nonfathers in 2005 ($n = 185$), men with higher PRL had more lifetime sexual partners ($P = 0.050$; Table 3, Fig. 3). In addition, men with greater PRL also showed a trend towards more sexual activity in the month before they were interviewed at baseline ($P = 0.060$; Table 3, Fig. 3). Because sexual activity is confounded by involvement in romantic relationships (Johnson et al., 2001), we adjusted both sets of models for partnering status. In these models (Table 3), PRL significantly positively predicted men's recent sexual activity ($P = 0.026$) and the positive relationship between PRL and lifetime sexual partners was borderline significant ($P = 0.060$).

DISCUSSION

Drawing on a large sample of men from the Philippines, we showed that partnered fathers had higher PRL than single nonfathers and childless men who did not live with their partners. Our results are generally consistent with findings from various bird and mammalian species showing that fathers who cooperate with mothers to raise young have elevated PRL. Fathers with infants also had higher PRL than fathers of older children. However, PRL did not predict who among the nonfathers at baseline had transitioned to first-time fatherhood 4.5 years later. Taken together, these findings suggest that PRL may rise after men become fathers at Cebu, particularly during early stages of their children's lives. In light of these results, we were surprised to find that nonfathers with higher PRL had more lifetime sexual partners and reported more sexual activity in the month before sampling. Thus, these outcomes reveal previously undescribed complexities in the relationships between PRL and men's mating effort, showing some broad commonalities with findings in certain other taxa (Rubin and Specker, 1992; Edwards et al., 1999; Leonard and Ferkin, 1999; Iwata et al., 2000; Preault et al., 2005; Toyoda et al., 2005). In total, our findings suggest the possibility that the relationships between PRL and male reproductive behavior can vary based on life history stage (e.g., nonfather vs. father).

To our knowledge, this is the first demonstration among human males that fathers have higher PRL compared with nonfathers. These findings differ from those of the only prior similar study, in which fathers were shown to have comparable baseline PRL to nonfathers in a sample of 43 Jamaican men (Gray et al., 2007). We also showed that fathers of infants had higher PRL compared with fathers of children older than 1 year, which suggests that within this specific cultural context it is the nature of one's current fatherhood status and related roles, rather than the simple presence or absence of any

offspring, that might matter for male PRL. Previous studies of T have shown that cultural differences in paternal caretaking roles influence whether fathers and nonfathers differ in T (Muller et al., 2009). Additional cross-cultural work will be necessary to clarify the extent to which variation in fathering roles contributes to variability in the relationship between PRL and fatherhood.

Although cross-species data generally indicate that when paternal PRL rises, it does so immediately prior to or in conjunction with the transition to fatherhood, the possibility exists that men with high PRL are simply more likely to become fathers, as opposed to fatherhood causing PRL to rise in human males. Findings from a previous cross-sectional study suggested that men might experience a transient increase in PRL prior to the birth of their children, with PRL then returning to baseline post-partum (Storey et al., 2000). Elsewhere it was found that new fathers' PRL remained unchanged throughout the first 6 months post-partum, although men's PRL prior to the birth of their children was not available (Gordon et al., 2010). In the present analyses, we found

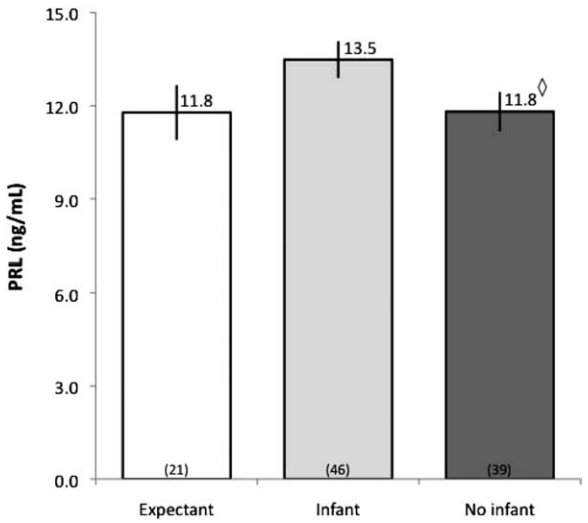

Fig. 2. PRL stratified according to whether men were expectant fathers [expectant], fathers to an infant-aged child (1-year-old or less) [infant], or fathers to older children [no infant]. Fathers of infants are the comparison group, $\diamond P = 0.054$. Models control for control for time of blood collection and usual wake time. Error bars indicate S.E.M.

that men who were fathers at baseline had higher PRL than nonfathers, whereas, among childless men, baseline PRL did not predict whether they subsequently transitioned to first-time fatherhood by follow-up, 4.5 years later. On the basis of this set of findings, it seems likely that the higher PRL measured among fathers at baseline occurred after their children were born, with the transition to fatherhood causing an increase in the hormone, rather than high PRL men being more likely to become parents. Our findings point to the need for future research to test this hypothesis using repeat measurement of PRL in men transitioning into fatherhood roles.

As has been discussed above, data from many species suggest that PRL helps facilitate paternal investment. However, little research has explicitly evaluated the converse, that low PRL predicts greater mating effort. Results from a single study of birds indicates this may be the case, as low PRL males were shown to invest more extensively in mating effort whereas high PRL males cooperated with females to raise young (Duckworth et al., 2003). Here, contrary to the notion that PRL is antagonistic to mating effort, nonfathers with higher PRL had more lifetime sexual partners and greater recent sexual activity. Notably PRL acutely increases after men orgasm (Kruger et al., 2002), but this effect is thought to be transient and is thus unlikely to account for our findings, though we cannot fully rule out the possibility that men had intercourse prior to our early morning clinic-based sampling.

Cross-species evidence provides insights as to possible pathways through which PRL might be related to greater mating effort. Among some seasonally breeding mammals PRL is crucial to the reactivation of the hypothalamic–pituitary–testicular axis prior to the onset of the mating season (Bartke, 2004), and PRL can also facilitate mating behaviors (Leonard and Ferkin, 1999). In at least one bird species, PRL also influences sexual ornamentation that signals how effectively males will perform as fathers (Preault et al., 2005). It is presently unknown whether human males express analogous PRL-sensitive cues of mate quality, as has previously been suggested for T-sensitive traits (Penton-Voak and Chen, 2004; Roney et al., 2006; Frederick and Haselton, 2007). However, PRL is known to stimulate the immune system, including the production and activity of T- and B-cells as well as antigen-presenting cells (Richards and Murphy, 2000), thus contributing to enhanced immunity, which may positively relate to men's attractiveness

TABLE 3. Predicting nonfathers' sexual activity at baseline from PRL (n = 185)

	Number of lifetime sexual partners[a]			
	Model 1	*P*	Model 2	*P*
PRL	1.05 (1.00, 1.10)	0.05	1.05 (1.00, 1.10)	0.06
Noncohabitating partnered[b]			1.55 (1.05, 2.30)	0.03
Cohabitating partnered[b]			2.49 (1.15, 5.40)	0.02
	Sexual activity in the last month[c]			
	Model 1	*P*	Model 2	*P*
PRL	1.09 (1.00, 1.19)	0.06	1.12 (1.01, 1.24)	0.03
Noncohabitating partnered[b]			4.61 (1.80, 11.78)	0.001
Cohabitating partnered[b]			55.92 (12.93, 241.86)	0.0001

[a] Values are IRR (95% CI) from negative binomial regression. PRL is adjusted for time of sample collection and usual wake time.
[b] Excluded comparison group: men who were not in a romantic relationship.
[c] Values are OR (95% CI) from ordered logistic regression. PRL is adjusted for time of sample collection and usual wake time.

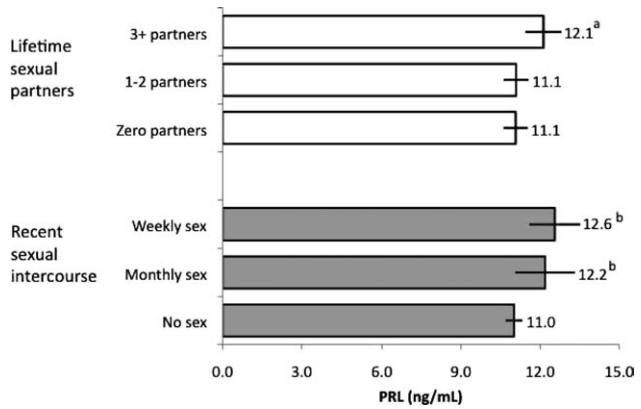

Fig. 3. PRL stratified according to nonfathers' self-reported number of lifetime sexual partners and their sexual activity in the month prior to the baseline interview. PRL is adjusted for time of blood collection and usual wake time. [a]Tertiles of lifetime sexual partners are presented for visual purposes. PRL positively predicted lifetime partners ($P = 0.050$), which was treated as a continuous variable in statistical analyses (see Table 3). [b]PRL predicted greater sexual activity in the last month ($P = 0.060$; see Table 3). Error bars indicate S.E.M.

(Shackelford and Larsen, 1999; Roberts et al., 2005). Another possibility is that, in this cultural context, PRL influences men's personality or behavior in ways that increase their attractiveness to potential mates. For example, in prior research it was found that men who had a higher interest in children were rated as more attractive by women (Roney et al., 2006) and men with higher PRL show more sensitive responses to child stimuli (Storey et al., 2000; Fleming et al., 2002). It bears mentioning that at baseline in our sample there were only two single fathers (who, being nonpartnered, may have been pursuing partnering/mating opportunities) and the partnered men did not report data on infidelity, which, together, precluded us from testing whether low PRL relates to greater mating effort or extrapair sexual activity among partnered men and fathers. This possibility merits further consideration. Overall, our findings suggest that hypotheses regarding PRL and mating effort could be fruitful for future studies to investigate.

Our study has limitations that warrant discussion. Because we did not collect bloodspot samples at both baseline and follow-up, we were unable to establish causality in the changes in PRL relative to men's transition to fatherhood. Additionally, using the average of PRL collected in several samples would have resulted in a more reliable estimate of each man's typical PRL level (Dabbs, 1990). Moreover, the PRL assay method used in this study is characterized by greater than ideal measurement error for low values of the hormone, as was also the case in the previous study in which this assay was validated (Gray et al., 2007). Our single bloodspot measurements and assay limitations do not introduce systematic bias, such as might increase the incidence of Type I errors, but merely reduce the reliability of our PRL measures and thereby limit our ability to detect significant relationships. To reduce the impact of these limitations, we collected samples at carefully controlled times in a clinical setting, standardized across all subjects, in a sample of men that exceeds the size of most prior studies of male socioendocrinology. The ability of our analyses to identify many statistically and biologically important relationships suggests that we had ample statistical power to test our hypotheses.

In conclusion, we found that fathers in committed romantic relationships had the highest PRL, differing significantly from single nonfathers and childless men who did not reside with their partners. However, we also found that childless men with higher PRL tended to have more lifetime sexual partners and had more recent sexual activity. Our results suggest that the role of PRL in human male reproductive strategies may be more complex than previously conceived and hint that the function of the hormone may shift as men move through different life history stages. Variable behavioral effects of PRL based on men's romantic and parenthood status could occur via complex interactions with other hormones and neurotransmitters, such as T, estrogen dopamine, serotonin, and oxytocin, and different patterns of receptor expression and hormonal binding thereof. These findings underscore the need for additional research designed to clarify the role of PRL in human male reproductive strategy and the neuroendocrine pathways through which it reaches expression.

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