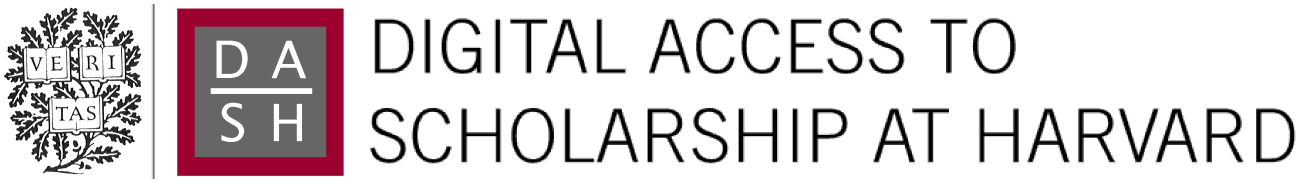

Marriage and motherhood are associated with lower testosterone concentrations in women

Marriage and motherhood are associated with lower testosterone concentrations in women.

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Abstract:

Testosterone has been hypothesized to modulate the trade-off between mating and parenting effort in males. Indeed, evidence from humans and other pair-bonded species suggests that fathers and men in committed relationships have lower testosterone levels than single men and men with no children. To date, only one published study has examined testosterone in relation to motherhood, finding that mothers of young children have lower testosterone than non-mothers. Here, we examine this question in 195 reproductive-age Norwegian women. Testosterone was measured in morning serum samples taken during the early follicular phase of the menstrual cycle, and marital and maternal status were assessed by questionnaire. Mothers of young children (age ≤3) had 14% lower testosterone than childless women and 19% lower testosterone than women who only had children over age 3. Among mothers, age of the youngest child strongly predicted testosterone levels. There was a trend towards lower testosterone among married women compared to unmarried women. All analyses controlled for body mass index (BMI), age, type of testosterone assay, and time of serum sample collection. This is the first study to look at testosterone concentrations in relation to marriage and motherhood in Western women, and it suggests that testosterone may differ with marital and maternal status in women, providing further corroboration of previous findings in both sexes.

Keywords: marriage; motherhood; testosterone; endocrinology

Introduction:

In males, testosterone plays a key role in modulating both mating effort and paternal investment. Physiologically, testosterone facilitates reproductive effort by promoting spermatogenesis and supporting the development of sexually dimorphic traits such as upper body muscle mass and increased stature (Bribiescas, 2001). Testosterone also contributes to reproductive effort behaviorally by promoting male-male competition and mate-seeking behaviors (Archer, 2006). The "challenge hypothesis", which emerged from avian research, posits that in species with biparental care, testosterone levels rise in contexts of male-male competition (particularly related to mating) and then decrease to facilitate care of young, dependent offspring (De Ridder et al., 2000; Nunes et al., 2000; Peters et al., 2002; Reburn and Wynne-Edwards, 1999; Wingfield et al., 1990; Ziegler, 2000). In humans, cross-sectional data suggests that testosterone levels are higher in: (1) unmarried men versus married men (Booth and Dabbs, 1993; Gray et al., 2002; Kuzawa et al., 2009); (2) uncommitted single men versus single men in committed relationships (Burnham et al., 2003; McIntyre et al., 2006; Sakaguchi et al., 2006; van Anders and Watson, 2006); and (3) non-fathers versus fathers (Gray et al., 2006b; Kuzawa et al., 2009; Muller et al., 2009). Longitudinal work supports this hypothesis as well. One cohort study found that testosterone levels were highest prior to marriage, declined during marriage, and then rose again if divorce occurred (Mazur and Michalek, 1998). Similarly, among single men without children, those who partnered and became fathers during a 4.5 year follow-up period had greater testosterone declines than those who remained single and childless (Gettler et al., 2011a).

Despite great interest in testosterone, marriage, and fatherhood in males, virtually no research has investigated this question in females and the role of testosterone in modulating female mating effort and maternal behaviors is poorly understood. Although testosterone clearly plays

a critical role in *some* components of female mating behavior, such as libido (Braunstein et al., 2005; van Anders et al., 2007), its contribution to other aspects of female mating and parenting effort are less apparent. Traditional evolutionary theory holds that intra-sexual mating competition is weak in human females and thus if testosterone primarily modulates intra-sexual mating competition, there should be little association with mating and maternal behavior (Bateman, 1948; Trivers, 1972). Nevertheless, research suggests that testosterone may be associated with mating behaviors in females of some species. In numerous avian species, female testosterone levels vary across the breeding cycle, typically peaking during ovulation and/or prior to the egg laying period, concurrent with the acquisition of mates and territory (Cristol and Johnsen, 1994 ; Gill et al., 2007; Ketterson et al., 2005; Osorno et al., 2010). In humans, several studies have found positive associations between testosterone and female aggression or competition, however it is unclear whether these findings are relevant within a mating context (Edwards and Kurlander, 2010; Harris et al., 1996). In fact, the limited research on female testosterone levels and mating status to date has been inconsistent. While some studies have found that among heterosexual women, being in a committed, monogamous relationship is associated with lower testosterone concentrations (Edelstein et al., 2011; van Anders and Watson, 2006), others have failed to find a relationship (Hooper et al., 2011).

Similarly, the research on testosterone and motherhood is in its infancy. Although there is some evidence that in marmosets, elevated testosterone is associated with decreased care-giving among mothers (Fite et al., 2005), to our knowledge, only one published study has examined testosterone, pair-bonding, and motherhood in humans (Kuzawa et al., 2010). In a cohort of Filipino women, waking salivary testosterone levels were lower in pair-bonded versus single women, and in mothers versus non-mothers. Mothers of young children, moreover, had significantly lower testosterone levels than mothers of older children, and only motherhood remained a significant predictor of testosterone levels in multiple regression models. While the

results are suggestive, the sample size (n=67) was small and population demographics skewed heavily towards very young mothers and included few married women without children. Furthermore, the study included women who were breast-feeding and/or using hormonal contraception, and testosterone levels were assayed from sample taken at different points in the menstrual cycle. Given these limitations, we attempted to replicate their study using stricter inclusion criteria in a second, demographically different population.

To this end, we used data from a Norwegian cohort study to test the following primary predictions: (1) testosterone levels are lower in married women than unmarried women; and (2) testosterone levels are lower in women with at least one child under age 3 than in women who do not have a child under 3 (*i.e*. are either nulliparous or have only older children). Among the mothers, furthermore, we predicted that the age of the youngest child and total parity would both be negatively associated with testosterone levels.

Materials and methods:

Study Population:

206 women participated in the Energy Balance and Breast Cancer Aspects (EBBA-I) study, based in Tromsø, Norway from 2000-2002. Participants were age 25-35 with self-described regular menstrual cycles and no use of hormonal contraceptives within the past six months. Women who had been pregnant or had breast-fed within the previous six months were excluded from participating in EBBA-I, as were women with known histories of infertility, gynecological disorders, or chronic illness. Women participated for one menstrual cycle and received 1000 Norwegian kroner (approximately $160 USD) on average to cover expenses associated with participation. Human subject approvals were obtained from Institutional Review Boards at all participating institutions. The study subjects and protocol have been previously described in greater detail (Furberg et al., 2005). For the current primary analyses, any woman who had

complete data on testosterone level, marital status, motherhood, age, height, and weight was included in the analysis (n=195). The 11 excluded women lacked serum data (n=6), questionnaire data (n=5), or both (n=2). Only mothers were included in secondary analyses (n=84).

Testosterone concentrations:

Fasting morning serum samples were collected during the early follicular phase (day 1-2) of the menstrual cycle and sample collection time was recorded to control for diurnal variation (Table 1). Fresh serum was allowed to rest for an hour after which it was centrifuged and immediately assayed at the University of Northern Norway (UNN) Department of Clinical Chemistry laboratory. Samples collected in the first few months of the study were assayed using Immuno1 from Bayer Diagnostics, after which the laboratory switched to the Elecsys 2010 assay from Roche Diagnostics. For both assays, the limit of detection was 1.0 nmol/L, which is high compared to those afforded by more recent female testosterone assay technologies. Average intra-assay variability was less than 5% for Immuno1, and inter-assay variability ranged from 4.0% for low pools to 3.7% for high pools. Average inter-assay variability was 6.5% for Elecsys 2010 and inter-assay variability ranged from 5.7% for low pools to 2.6% for high pools.

Motherhood and marriage:

At baseline, women completed a questionnaire which included marital status (single, married/living as married, widowed, divorced/separated, or other). Women who were divorced or separated (n=6) were grouped with single women for these analyses. No women reported being widowed or "other". For brevity, in this paper, we refer to the women who reported being "married/living as married" as simply, "married". Subjects also reported whether they had children or not. The birthdates of those children were collected during follow-up interviews in fall 2004 and using that information, motherhood status, parity, and age of youngest child at

baseline were calculated. From this data, two additional variables were derived: (1) youngest child ≤age 3 at baseline; (2) youngest child >age 3 at baseline. Age three was chosen as a cutoff for two reasons: (1) it tends to mark a transition away from complete physical dependence on parental care and increased self-sufficiency; and (2) the sample sizes of the two resulting groups were sufficient to make inferences about the relationship between maternal testosterone and motherhood.

Assessment of additional covariates:

Based on previous work in other populations suggesting that energetic factors such as energy intake (Hainer et al., 2001), physical activity (Enea et al., 2011), and sleep (Andersen et al., 2011) may affect testosterone levels in women in some contexts, we included several additional variables in our secondary analyses. In the questionnaires, subjects reported typical hours of sleep per night as well as typical physical activity level in the past year (on a scale of 1-4; 1= sedentary or low activity, 2=moderate activities at least 4 hours per week, 3= hard activities to keep fit for at least 4 hours per week; 4=hard training or exercise for competition several times per week). Assessment of physical activity levels in the EBBA-I subjects is described in greater detail elsewhere (Emaus et al., 2008). Because very few subjects reported high activity, groups 3 and 4 were combined in the analyses. In addition to the questionnaires and clinical visits, subjects complete detailed food diaries for seven days across the cycle (days 3-6 and days 21-23), which were then scored by nutritionists at the University of Oslo to determine typical energy intake. Height and weight at baseline were recorded using standard techniques (Furberg et al., 2005) and body mass index (BMI) was then calculated as weight (kg)/ height (m^2).

Statistical analysis:

All analyses were performed first with SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA) and repeated in R by a second, independent analyst to confirm results (R Version 2.9.0). Because testosterone values are typically non-normally distributed, we fit our models using both the raw

and log-transformed testosterone values. The log-transformed data fitted better the linear regression model assumptions which included normality and homoscedasticity of the errors and consequently was used for the current analyses. Our primary model (Model 1) was a linear regression model of log(testosterone) as a function of maternal age, BMI, time of testosterone sample collection, marital status (married or not), testosterone assay type (Immuno1 vs. Elecsys), and age of the youngest child. Age of youngest child was coded with two dummy variables to distinguish three groups: women with no children, women with a child ≤ 3 years and women with children all > 3 years. In a separate model (not shown), we also considered the interaction between marital status and age of youngest child. Because the limit of detection (LOD) of the assay was 1.0 nmol/L, all samples with testosterone levels below the LOD were assigned a value of 1.0 nmol/L for analysis. Sensitivity analyses were subsequently conducted to test whether the results differed when we instead assigned a value of LOD/ √2 or LOD/2 to testosterone concentrations below the sensitivity limit.

A secondary linear regression model (Model 2) was fit which was similar to Model 1 but with adjustment for three additional energetic variables: average daily energy intake, typical physical activity level, and typical hours of sleep per night. Finally a pair of models (Models 3 and 4) was fit for mothers only, analyzing testosterone levels in relation to parity and age of youngest child (treated continuously). Parity was modeled both as strictly ordinal (1-5 children) and as groups (1, 2, or 3+ children) and the second of the two models included the energetic variables. All variables were chosen *a priori* and included in the final models, even if not significant. Model assumptions of linearity between covariates and outcome and normally distributed error with constant variance were checked. For each model, we identified outliers and influential points and reran the models excluding those subjects. All p-values reported are two-tailed, with an alpha level of p=0.05.

Results:

Demographic measures:

Table 1 displays demographic data stratified into three groups based on motherhood status: women with no children, women with a child age ≤3 years and women with children all age >3 years. The mean age of the women was 30.9 years (range 25.0-35.9), however, non-mothers were significantly younger than both groups of mothers. Fewer than half of non-mothers were married, moreover, compared to over 80% of mothers. Mothers of young children reported significantly higher average daily energy intake than mothers of older children, but neither group differed significantly from non-mothers. Mothers of young children reported getting significantly fewer hours of sleep on a typical night than non-mothers. The three groups showed no significant differences in height, weight, BMI, age at menarche, physical activity level, or time of serum sample collection.

Association of testosterone with marriage and motherhood:

Results from our primary model (Model 1) show that after adjusting for covariates, having a child age ≤3 is associated with lower testosterone levels and there is a trend towards marriage being associated with lower testosterone levels as well (p=0.10) (Table 2). Mothers with a child age ≤3 had lower testosterone levels than both women without children (p=0.02) and mothers of older children (p=0.002; not shown). Reflecting well-documented diurnal rhythms, time of serum sample collection was also a significant predictor of testosterone levels (p=0.01). The type of testosterone assay used was a highly significant predictor of testosterone levels, but neither BMI nor age was associated with testosterone levels. In a separate model, the interaction between marital status and age of youngest child (no children, child age ≤3, and child age >3) was not significant (p=0.28 for two degree of freedom test). Figure 1 shows mean (log) testosterone values and 95% confidence limits by marital and motherhood status from this interaction model, after adjustment for age, BMI, type of testosterone assay, and time of sample

collection. In our larger model (Model 2), we further adjusted for average daily energy intake, physical activity level, and hours of sleep per night, however none of these variables was a significant predictor of testosterone levels (Table 2).

Predictors of testosterone levels among mothers:

In Model 3 we investigated the influence of parity and age of youngest child (entered as a continuous variable) on testosterone concentrations in mothers, further adjusting for marital status, age, BMI, type of testosterone assay, and time of serum sample collection (Table 2). Age of youngest child was highly significantly and positively associated with testosterone levels ($p=0.009$) among the 84 mothers in the sample. Time of sample collection was no longer a significant predictor of testosterone levels. The results were unchanged regardless of whether we considered parity continuously (1-5 children) or by group (1, 2, or 3+ children), and the latter is presented in Table 2. When we further adjusted for the energetic variables (caloric intake, activity level, and total sleep), results were very similar and none of the energetic variables was a significant predictor (Model 4, not shown).

Model assumptions were reasonable when using testosterone on the logarithmic scale. The exclusion of outliers and influential points did not change the estimates for our covariates by more than 5%, therefore all subjects were retained in the models. In sensitivity analyses assigning a testosterone value of LOD/$\sqrt{2}$ (rather than LOD) to samples below the sensitivity limit (not shown), compared to the original models, two differences emerged. First, our estimates for the association between marriage and testosterone concentrations became larger (i.e. less conservative), however due to increased standard errors, our p-values became larger as well. Second, our estimates for the association between having a young child and testosterone concentrations became larger and the p-values became smaller, suggesting that our original estimate and p-values were a conservative assessment of that relationship. These

findings were even more pronounced when we reran the sensitivity analyses instead assigning a testosterone value of LOD/2 to samples at the sensitivity limit (not shown).

Discussion:

We hypothesized that marriage and motherhood would be associated with lower testosterone levels, and that testosterone levels would be particularly low among mothers of young children. In our 195 reproductive-age Norwegian women, we found differences in the predicted direction, namely married women showed a trend towards lower testosterone levels than unmarried women and mothers of children age ≤3 had significantly lower testosterone concentrations than women with older children. To our knowledge, the results of the current study are the first of their kind in Western women of older reproductive age and they confirm and extend Kuzawa et al. (2010)'s previous work. Both studies found lower testosterone levels (32% lower testosterone in the Filipino study vs. 14% in the current study) among mothers of young children compared to women who did not have young children, despite considerable differences in culture and sociodemographics. In general, the Filipino women were younger (age 20-22 vs 25-35 for our study population), many did not head their own households, and many were still in school, compared to our older, more established population.

Our findings also agree with similar research in males, in which fatherhood is frequently, though not always, associated with lower testosterone levels (Gettler et al., 2011a; Gray et al., 2004; Gray et al., 2002; Gray et al., 2006b; Kuzawa et al., 2009; Muller et al., 2009). In males, lower testosterone levels among fathers may be related to direct involvement with the daily care of children and/or the larger societal context. For instance, in two neighboring, traditional Tanzanian populations, one with high levels of direct paternal care (the Hadza), and the other with low levels of direct paternal care (the Datoga), found that only the high-investing Hadza experienced testosterone changes related to paternal status (Muller et al., 2009). Indeed,

cross-cultural differences in child-rearing norms may account for mixed findings on the relationship between paternal testosterone levels and the age of the youngest offspring (Gray, 2003; Gray et al., 2002; Gray et al., 2006a; Muller et al., 2009) or the amount of direct paternal investment (Gettler et al., 2011a; Gray et al., 2002). Future research on this question in women should more closely examine quantity and quality of direct parental care in relation to maternal testosterone levels, particularly longitudinally.

Evolutionarily, the low testosterone levels found in parents of young children may represent a trade-off from investment in mating effort to investment in parenting effort. While testosterone facilitates intra-sexual competition for mates, libido, and aggression, those behaviors may be unnecessary for, or even incompatible with, effective parenting, and thus there may have been natural selection for lowered testosterone levels coincident with increased parental investment (Gray and Campbell, 2009). Alternatively, low testosterone levels in parents of young children could be a by-product of the high energetic demands of caring for a small child, given that some types of acute or chronic stress may be associated with reduced testosterone levels (Bribiescas, 2001). To address this possibility, we included average energy intake, physical activity level, and hours of sleep per night in a larger model, however none of these variables predicted testosterone levels. Other studies have similarly failed to identify energetic variables as important mediators in the relationship between marriage, parenting, and testosterone (Gettler et al., 2011b; Gray et al., 2002; Kuzawa et al., 2010), thus we suggest that the low testosterone levels seen in mothers of young children are unlikely to be attributable to energetic stressors.

One strength of our study is that unlike previous work, we used multivariable modeling to examine how the interaction between marital and parental status was associated with testosterone levels. Among childless women and mothers of older children, marriage tended to be associated with lower testosterone levels. Interestingly, among mothers of young children,

the opposite pattern was present, whereby marriage was associated with higher testosterone levels. Although the sample size for that sub-population was small and the results were not statistically significant, it suggests that in those women, there may be an extreme trade-off between intense investment in parental care and minimal investment in mating effort. Because human children are so altricial and our species' evolved family structures tend to facilitate biparental care, single mothers of young children represent an evolutionarily novel situation. Notably, single mothers of older children did not have lower testosterone levels, possibly indicating that once dependent care becomes less demanding, single mothers can again invest in mating effort. It is also possible that elevated psychosocial stress commonly reported among single mothers (Avison et al., 2007; Franz et al., 2003) may manifest itself in physiological changes that reduce testosterone and other markers of mating effort, though we know of no research that directly addresses this question.

Another notable strength of our study was our inclusion criteria. In Kuzawa et al. (2010), there was cross-subject variation as to when, during the menstrual cycle, testosterone concentrations were measured, and some subjects were using oral contraceptives or breast-feeding at the time of participation. Although the authors considered these important covariates during analysis, our study design reduced or entirely eliminated these potential sources of variation in testosterone levels. Because the original EBBA-I study was designed to look at natural variation in hormone levels across the cycle, women were excluded if they had been pregnant or breast-feeding within the last six months. Similarly, because subjects were ineligible if they had used hormonal contraception within the previous six months and all serum samples were taken within a small window of the follicular phase of the menstrual cycle, thus we can rule out those variables as potential confounders in our analyses.

Issues with the testosterone assays used present several limitations for our study, unfortunately. First, our testosterone level were measured in serum, rather than saliva, as in many related studies (Gray et al., 2002; Kuzawa et al., 2010) and thus reflect free (biologically active) hormone levels as well as the bound, inactive fraction. Ideally, we would prefer to examine both the free and the total levels, as can be done with current assay technologies, however it is worth noting that we found the same relationships with total serum testosterone that have previously been found in studies analyzing salivary (free) testosterone and previous work has found similar relationships between testosterone and fatherhood regardless of whether testosterone was measured in saliva or serum (Kuzawa et al., 2009).

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Second, the high LOD of the testosterone assay (1.0 nmol/L) meant that the range of values below 1.0 nmol/L was truncated and all women with testosterone levels at or below that level were assigned a value of 1.0 nmol/L (which actually represents the highest possible concentration that they could have). Because women with young children were disproportionately represented among subjects with testosterone levels at the LOD, it is likely that their group mean is even lower, suggesting that our findings may actually be an underestimate of the true relationship between having a young child and testosterone levels. When we conducted sensitivity analyses assigning LOD//√2 or LOD/2 (rather than simply the LOD, 1.0 nmol/L) to samples with values below the LOD, we found that the estimate of the association between marriage and testosterone grew stronger, but the p-value increased due to increased standard errors. The association between having a young child and testosterone levels similarly strengthened and became more significant, suggesting that we are likely underestimating the strength of the relationship. Therefore, we predict that with the more sensitive assays available today, we would see an even greater effect size between testosterone levels and having a young child. Finally, the interpretation of our results is complicated by the fact that the clinical laboratory switched from the use of one testosterone

391 assay to a second one partway through the study. Although the correlation of testosterone
392 levels measured in samples run in parallel on the two assays was high (particularly among high
393 testosterone samples), it still represents an additional source of error and variation.
394 Nevertheless, as shown, there were still associations between testosterone and motherhood
395 after adjusting for testosterone assay type in our models.
396
397 In our study, one potential source of misclassification bias is based on marital status. First, our
398 questionnaire did not differentiate between married and *samboer*, a legally recognized
399 cohabitation status which is best translated as "living as married". Unlike in some Western
400 nations, in Norway, there is no stigma to this designation and it is common for *samboer* couples
401 to have children and never formally wed. Based on that, we considered women who were
402 married or *samboer* as a single group, indicative of a highly committed relationship. If women
403 who were *samboer* were actually more similar to unmarried women, their exposure
404 misclassification would likely have been non-differential and biased our results towards the null.
405 In addition, we do not have data on sexual activity or sexual fidelity in any of the women studied
406 (married or single), although results from other studies suggest they may be useful to consider
407 in relation to testosterone concentrations in women (van Anders et al., 2007). Additional
408 research is needed to disentangle the associations between testosterone, relationship status,
409 and sexual activity in women.
410
411 Because our study was cross-sectional, we cannot determine the direction of causality or reject
412 the possibility that women with high testosterone levels are less likely to marry and become
413 mothers. Certainly, some clinical conditions characterized by supranormal testosterone levels,
414 such as polycystic ovary syndrome, may be associated with lower fecundity (Mellembakken et
415 al., 2011), however our inclusion criteria excluded women likely to have a hormonal or serious
416 medical condition. To our knowledge, no study has examined whether testosterone levels in

417 women within the normal range are related to their likelihood of marrying or conceiving. Our
418 finding, furthermore, that testosterone levels were only lower in mothers of young children
419 suggests that the suppression is temporary ("state", not "trait") and that as children age beyond
420 their peak dependent years, a mother's testosterone levels may increase.
421
422 Our findings agree with and extend previous research in females and the larger body of work in
423 males. Nevertheless, it remains uncertain as to whether the associations between testosterone,
424 marriage, and parenting in females represent adaptations shaped by natural selection (as has
425 been argued persuasively in males) or whether there are alternative explanations. Limited
426 research in other species has found that high testosterone levels may be associated with
427 territorial defense by females, but reduced care of offspring, supporting an adaptive explanation
428 (Fite et al., 2005; Moller et al., 2005). It is also possible that this relationship in females emerged
429 as a by-product of selection for testosterone-mediated modulation of mating and parenting effort
430 in males, although testosterone levels co-vary in males and females in some, but not all,
431 species (Ketterson et al., 2005).
432
433 The fact that testosterone production in the two sexes differs further complicates the
434 comparison. In males, the testes produce the majority of testosterone with negligible adrenal
435 contributions, whereas in females, the ovaries and adrenals contribute approximately equally
436 (Longcope, 1986). Thus in men, differences in testosterone associated with pair-bonding and
437 fatherhood are presumed to be related to down-regulation of testosterone production by the
438 testes (Kuzawa et al., 2009). While it may be most obvious to attribute differences in circulating
439 testosterone levels among females to homologous differences in ovarian function, differences in
440 adrenal androgen production and activity may also be implicated. A comparison of luteinizing
441 hormone (LH) and adrenocortiocotropic hormone (ACTH) concentrations in relation to marriage
442 and motherhood might help to resolve this question. If levels of LH, but not ACTH, differ by

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443 marital or motherhood status, it would provide evidence for differences in gonadal testosterone
444 production, possibly as a by-product of selection in males. Conversely, differences in ACTH
445 would implicate differences in adrenal androgen production, possibly indicating an adaptive
446 neurobiological response to pair-bonding and parenting. Acute variation in ACTH concentrations
447 in relation to stress and the pulsatile nature of ACTH release may make such a comparison
448 difficult or impractical, however (Carnes et al., 1988; Lennartsson et al., 2012; Van Cauter et al.,
449 1981). It may be similarly important to consider the role that psychological mechanisms play in
450 modulating the relationship between testosterone, marriage, and motherhood in females.
451 Several studies have examined maternal behaviors in relation to testosterone, finding that
452 among new mothers, higher testosterone is associated with less affectionate behavior towards
453 their infants (Fleming et al., 1997), more mood disturbances (Buckwalter et al., 1999), and
454 higher scores on depression and anger inventories (Hohlagschwandtner et al., 2001). In young
455 women, furthermore testosterone administration increased neural response to (non-related)
456 infant crying (Bos et al., 2010).
457
458 Finally, we cannot rule out the role that other hormones involved in the co-regulation of female
459 reproductive function may play in driving this relationship. It will be important for future research
460 to consider not only the associations between testosterone, marriage, and motherhood, but also
461 the role of other hormones implicated in mating and nurturing behavior, such as oxytocin
462 (Feldman, 2012; Feldman et al., 2007; Gordon et al., 2010; van Anders et al., 2011), cortisol
463 (Fleming et al., 1997; van Bakel and Riksen-Walraven, 2008), and the ovarian steroids,
464 estradiol and progesterone (Fleming et al., 1997; Rosenblatt, 1994). Indeed, given that much
465 ovarian testosterone is aromatized to estrogen, it is possible that the upregulation of
466 testosterone production associated with mating effort is, in fact, due to selection for increased
467 estrogen production. Similarly, the lower testosterone we have found among women with young
468 children could represent selection for temporary reproductive suppression through decreased

estrogen levels. This possibility may be further explored by examining estrogen levels in relation to maternal status.

In conclusion, our study showed that among Norwegian women, both marriage and motherhood were associated with lower testosterone levels and that having a child age ≤3 was a particularly strong predictor of low testosterone levels. Future work should address longitudinal changes in female testosterone levels over time, the biological mechanisms underlying the relationships, and the association between testosterone and quantity and quality of direct maternal care.

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Table and Figure Captions:

Table 1. Characteristics of the study population (mean±S.D.).

Table 2. Multiple regression models predicting testosterone levels. Testosterone data were log-transformed for the regression, thus units are expressed as log(nmol/L) with 95% CI indicated. Results in bold indicate significance at α=0.05.

Figure 1. Comparison of mean log(testosterone) levels in log(nmol/L) by marital and motherhood status. The error bars show the upper 95% confidence interval for the predicted response among subjects with the Immuno1 testosterone assay and the mean values of BMI, age, and time of serum sample collection.

Figure 2. Relationship between log(testosterone) and age of youngest child. Data points are observed values. The lines show the slope and a 95% confidence interval for the slope from the regression model adjusted for marital status, time of serum sample collection, body mass index, age, parity, and testosterone assay type.