

Diurnal testosterone variability is differentially associated with parenting quality in mothers and fathers☆



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ABSTRACT

Previous studies on the relation between testosterone (T) levels and parenting have found ample evidence for the challenge hypothesis, demonstrating that high T levels inhibit parental involvement and that becoming a parent is related to a decrease in T levels in both mothers and fathers. However, less is known about the relation between T levels and more qualitative aspects of parenting. In the current study we examined basal T levels and diurnal variability in T levels in relation to mothers' and fathers' parenting quality. Participants included 217 fathers and 124 mothers with two children (3 and 5 years of age). Evening and morning salivary T samples were analyzed with radio-immunoassays to determine circulating T levels. Parental sensitivity (i.e., child-centered responsiveness) and respect for children's autonomy were observed during free play in the family home. The results showed that higher evening T levels in mothers were associated with more sensitivity to the oldest and youngest child. Diurnal T variability was more consistently associated with parenting behavior towards their children than basal T levels. For fathers, more diurnal variability in T was associated with *more* sensitivity and *more* respect for autonomy with their youngest children. For mothers, more diurnal variability in T was associated with *less* sensitivity to both children and *less* respect for the youngest child's autonomy. These findings suggest that the T system might act differently in relation to parenting behavior in males and females.

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Introduction

The relation between testosterone (T) and behavior is often presented within a trade-off framework that contrasts high T levels accompanied by a focus on competitive challenges and mating with low T levels accompanied by a focus on parenting (Van Anders et al., 2012). According to the "challenge hypothesis" the association between T and parenting is reciprocal, with high T levels inhibiting parenting, and cues associated with children, child care, or parenting being related to a decrease T levels in both mothers and fathers. A number of studies have found support for the challenge hypothesis (Berg and Wynne-Edwards, 2001; Gettler et al., 2011b; Kuzawa et al., 2010; Mascaro et al., 2013; Wingfield et al., 1990), but it should be noted that most of these have involved only male participants.

Some studies provide evidence for the proposition that variations in basal T levels are associated with variations in paternal involvement and quality of involvement. For example, men with lower T levels, compared with men with higher T levels, held test baby

dolls longer (Storey et al., 2000), showed more affectionate touch, gaze, and vocalization during father-child interaction (Weisman et al., 2014), more often had children (Gray et al., 2002), and provided more direct care for their children and more economic support for the family (Alvergne et al., 2009).

Another study has shown that T levels change in response to parenting or child cues, indicating that fatherhood, and more involvement in child care and time spent with children were associated with subsequent lower T levels in fathers (Gettler et al., 2011b). To date, only two studies examined the relation between T levels and parenting behavior in women (i.e., comparing T levels of mothers, non-mothers, married, and non-married women). Both studies found – in line with the challenge hypothesis – that marriage and motherhood were associated with lower levels of circulating T in women (Barrett et al., 2013; Kuzawa et al., 2010). These studies suggest that circulating T is important for parenting behavior in both mothers and fathers.

However, according to the Steroid/Peptide Theory of Social Bonds the association between T levels and parenting behavior might be more complicated than proposed by the challenge hypothesis. The Steroid/Peptide Theory of Social Bonds assumes that "only those infant/parent contexts that involve nurturance will decrease testosterone; those that involve competitions (real or imagined) will increase testosterone" (Van Anders et al., 2012, p. 31). Several studies have shown that baby cries, that can be considered as a challenge, indeed

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increase T levels in men (Fleming et al., 2002; Storey et al., 2000). However, a recent study has shown that baby cries do not always lead to an increase in T levels (Van Anders et al., 2012). It was demonstrated that baby cries were associated with decreased T levels in men when cries could be terminated by participants' nurturing responses. In contrast, when they were not able to respond with nurturing behaviors, they showed increased T levels. In addition, the administration of T in women enhances, rather than suppresses, neural responsivity to baby cries in women (Bos et al., 2010), probably by increasing oxytocin levels through its metabolite estradiol.

Most studies on levels of circulating T in relation to parenting behavior have focused on basal levels of T and do not capture the diurnal variability in the production of T levels. Just like individual differences in basal T levels, individual differences in T variability over the day can be viewed as trait-like biological predispositions (Granger et al., 2003), possibly related to individual differences in parenting behavior. There is some evidence that diurnal variation in T can be considered a trait-like feature, as the diurnal rhythm of T shows individual differences (Axelsson et al., 2005) which have been found to be fairly stable across the adult male lifespan, even though the basal T production declines with age (Diver et al., 2003; Touitou and Haus, 2000).

The importance of examining diurnal change in T in relation to parenting is further supported by the large literature on diurnal cortisol rhythms. A flattened diurnal cortisol rhythm is predictive of negative behavioral and psychosocial outcomes in both children and adults (e.g., Burke et al., 2005; Marceau et al., 2014). The cortisol (hypothalamus–pituitary–adrenal, HPA) system is highly intertwined with the testosterone (hypothalamus–pituitary–gonadal, HPG) system, as also evidenced by the similar diurnal rhythms of cortisol and T. In general, the diurnal rhythm of T is characterized by highest T levels in the morning, steeply declining levels before noon, followed by a slower decline in the afternoon and early evening, reaching the lowest levels in the evening (Booth et al., 2006; Cooke et al., 1993). There is evidence that the diurnal rhythm of T is more pronounced in males compared with females (Granger et al., 2002).

To our knowledge, there are only two studies that have examined T variability in relation to behavioral outcomes. A study among Japanese adult men showed that *less* diurnal variation in T was associated with mostly negative outcomes; type A personality, more perfectionism, being a workaholic, excessive self-monitoring, and shorter sleep duration, but also less sensation seeking (Sakaguchi et al., 2006). Moreover, a study examining the association between diurnal T rhythm and problem behavior in adolescents provides novel evidence that T variability is differently linked to behavior in males and females (Granger et al., 2003). For females, *more* diurnal variability in T was related to higher levels of disruptive behavior problems, whereas for males *less* diurnal variability in T was associated with higher levels of anxiety, depression, and attention problems. Although this study was conducted with adolescents, it provides first evidence that in males *less* diurnal variability in T might be associated with non-optimal behavior, whereas in females *more* diurnal variability in T might be associated with non-optimal behavior.

Unfortunately, the relation between diurnal T variability and parenting behavior has received little attention. To date, one study showed that fathers who practiced same-surface cosleeping had a significantly greater diurnal decline in T from waking to bedtime compared with fathers who slept solitarily (Gettler et al., 2012), suggesting a relation between parental involvement and diurnal T variability in fathers. Next to parental involvement, parental sensitivity and respect for autonomy are important aspects of parenting in early childhood. Sensitivity refers to the adult's ability to notice child signals, to interpret these signals correctly, and to respond to them promptly and appropriately (Ainsworth et al., 1974). Many studies emphasize the importance of parental sensitivity for positive early child development across several developmental domains (e.g., Bakermans-Kranenburg et al., 2003; Biringen et al., 2014; Lucassen et al., 2011; Tamis-LeMonda et al.,

2004). Respect for autonomy refers to the parent's ability to refrain from behavior that is over-directing, over-stimulating, or interfering in the child's activities (Biringen et al., 2014). A lack of respect for the child's autonomy has been associated with non-optimal outcomes during early childhood, such as externalizing behaviors and lower academic achievement (e.g., Cabrera et al., 2007; Egeland et al., 1993; Ispa et al., 2004). As discussed above, cosleeping has been found to be associated with larger diurnal declines in T (Gettler et al., 2012), but to our knowledge there are no studies relating T levels or T variability to the overall constructs of sensitivity or respect for children's autonomy.

In the current study we examine basal T levels and diurnal variability in T levels, measured in saliva, in relation to mothers' and fathers' sensitivity and respect for autonomy towards their children in early childhood. First, based on the challenge hypothesis, we expect lower basal T levels to be associated with more sensitivity and respect for autonomy in both mothers and fathers. Second, we expect a relation between diurnal variation in T and parenting quality. There is some evidence that T variability is related to personality characteristics and problem behavior in adolescents and adults, but the direction of the association between T variability and behavior is not clear and might be different for males and females (Granger et al., 2003; Sakaguchi et al., 2006). Therefore, the association between T variability and parenting quality in mothers and fathers will be examined in an explorative manner.

Method

Sample

This study is part of the longitudinal study 'Boys will be Boys?' examining the influence of mothers' and fathers' gender-differentiated socialization on the socio-emotional development in boys and girls in the first years of life. The current paper reports on data from the third wave of the study, during which saliva samples were collected.

Families with two children were selected from municipality records in the Western region of the Netherlands. Families were included if the second-born child was around 12 months of age and the firstborn child was approximately two years older. For more information about the selection procedure, see Endendijk et al. (2013). Of the 1249 eligible families 31% were willing to participate ($n = 390$). At the third wave, 18 families no longer participated because of problems in the family ($n = 3$), moving abroad ($n = 5$), considering the home visits too demanding ($n = 7$), or because they could not be reached by phone or mail ($n = 3$). Because a large number of mothers were excluded for the current study due to pregnancy or contraceptive use ($n = 109$), we chose to report on fathers and mothers separately to maximize the sample sizes.

For the current study, fathers with missing data ($n = 141$) and use of medication known to affect hormone levels (e.g., anti-depressants, anti-psychotics, $n = 14$) were excluded, resulting in a sample of 217 fathers. Excluded fathers were not different from included fathers in terms of age ($p = .31$), educational level ($p = .44$), or degree of urbanization of residence ($p = .89$). Within the final group of fathers, 49% of the oldest children and 53% of the youngest children were boys. At the time of wave 3, the youngest children were 3.1 years old ($SD = 0.1$) and the age of the oldest children ranged from 4.5 to 5.7 years ($M = 5.0$, $SD = 0.3$). The fathers were aged between 28.0 and 55.3 years ($M = 38.6$, $SD = 5.1$). With regard to educational level, most fathers finished academic or higher vocational schooling (73%). During the study, five fathers (2%) got divorced, and in 16% of the families a third child was born ($n = 35$). Analyses with and without these families yielded similar results, so these families were retained in the current data set.

To obtain the sample of mothers for the current study, we excluded mothers with missing data ($n = 128$) and use of medication that affects hormone levels ($n = 11$). In addition, mothers who were pregnant ($n = 18$) or used contraceptives ($n = 91$) were excluded, resulting in a final sample of 124 mothers. Excluded mothers were not different

from included mothers in terms of age ($p = .57$) or degree of urbanization of residence ($p = 1.00$), but were slightly higher educated than the included mothers ($p < .05$). Within the final sample of mothers, 53% of the oldest children and 53% of the youngest children were boys. At the time of wave 3, the youngest children were 3.1 years old ($SD = 0.1$) and the age of the oldest children ranged from 4.5 to 5.6 years ($M = 5.1$, $SD = 0.3$). The mothers were between 27.7 and 47.7 years of age ($M = 35.9$, $SD = 4.2$) and most of them had finished academic or higher vocational schooling (72%). In 20% of the families a third child was present ($n = 25$). Within the samples of mothers and fathers, 106 were from the same families.

Measures and procedure

Each family was visited twice; once with the mother and the children and once with the father and the children, with an intervening period of about two weeks. The order in which mothers and fathers were visited and interacted with the oldest and youngest child was counterbalanced between families. Before the first home-visit both parents were asked to individually complete a set of questionnaires (e.g., about the child's temperament, internalizing and externalizing behavior, empathy). During the home visits parent-child interactions and sibling interactions were filmed (to assess behaviors such as child prosocial behavior and parental sensitivity, control) and both children and parents completed computer tasks assessing gender stereotypes. For more information about the procedure, see Endendijk et al. (2013). To measure parental T levels, parents were asked to collect two saliva samples (i.e., passive drool) on a weekday between the mother and father visits, the first sample before going to bed (PM) and the second sample at waking (AM). Parents also filled out a questionnaire to establish basic background information associated with hormone levels (e.g., weight, pregnancy, and physical activity). Saliva samples were stored in the parent's own freezer until pick-up and were then stored at -80°C until analysis.

Parental T levels

Salivary samples were analyzed at the endocrinology laboratory at Utrecht Medisch Centrum (Utrecht, the Netherlands). T levels in saliva were measured in duplicate using an in-house competitive radio-immunoassay employing a polyclonal anti-testosterone-antibody (Dr. Pratt, AZG 3290). As a tracer following chromatographic verification of its purity, $[1,2,6,7\text{-}^3\text{H}]\text{-Testosterone}$ (NET370250UC, PerkinElmer) was used. The lower limit of detection was 20 pmol/L. Inter-assay variation was 10.5–8.3% at 70–480 pmol/L respectively ($n = 33$). To obtain a measure of T variability in parents, we calculated the ratio of diurnal change as follows: $((\text{T evening} - \text{T morning}) / \text{T evening}) * -1$.

Parental sensitivity and respect for autonomy

The fourth edition of the Emotional Availability Scales (EAS; Biringen, 2008) was used to measure parental sensitivity and respect for autonomy towards their children during a semi-structured free play session. Each dyad received a bag with toys and was invited to play for 8 min. This relatively unstructured home setting is similar to play time between parent and child in a natural setting. Therefore, the observed parental behaviors are likely to be representative of parents' natural behavior (Gardner, 2000). For more information about this measure, see Hallers-Haalboom et al. (2014). Two groups of in total nine coders rated the videotapes on the EAS dimensions. All groups completed a reliability set ($n = 60$), with at least 42% overlap between the two sets. Intercoder reliability was adequate, the intraclass correlation coefficients (single measure, absolute agreement) for sensitivity ranged from .71 to .92 and for respect for autonomy from .71 to .92. All dyads within the same family were coded by different coders to guarantee independency among ratings. During the coding process, the first 100 videotapes of every coder were coded independently by

separate coders and regular meetings were organized to prevent coder drift.

Data-analysis

All variables were inspected for possible outliers, defined as values of more than 3.29 SD above or below the mean (Tabachnick and Fidell, 2012). Outliers were found for parental T levels ($n = 6$) and paternal respect for autonomy ($n = 1$). The outlying scores were winsorized to decrease the difference between the outlier and most extreme value that was not yet an outlier (Tabachnick and Fidell, 2012). All variables were normally distributed.

All analyses were done for morning and evening T levels, and diurnal variability in T. Prior to the analyses, correlations were inspected between T levels and possible confounding factors (i.e., hours of care for children, working hours, sleep quality on night before saliva collection, third infant child in the family). To control for the effects of age and weight on parental T levels, residual scores were computed. For those fathers and mothers who were couples, paired sample t -tests were conducted to examine differences between fathers' and mothers' T levels, and Pearson correlation coefficients were computed to examine associations between fathers' and mothers' T levels, as well as their relations with parenting quality. One bivariate outlier was detected in the sample of fathers and this case was deleted from further analyses.

To take into account that mothers and fathers are nested within couples, multi-level analyses (i.e. SPSS mixed model analyses) were also conducted for the couples on which we had complete data for both mothers and fathers ($N = 106$). All predictors were grand-mean centered. In these analyses, effects of parents' own T levels (and diurnal change in T) and effects of the partner's T levels (and diurnal change in T) were modeled, as both can account for the statistical interdependence between mothers and fathers. In addition, interactions between parent sex and parent's own or partner's T levels were tested.

Results

Preliminary analysis

All T values (mothers: 10–559 pmol/L, fathers: 48–1040 pmol/L) fell within the range of values reported in other studies measuring T in saliva (e.g., Dabbs and de La Rue, 1991; Gettler et al., 2011b; Gray et al., 2002; Kuzawa et al., 2010). Hours of caring for children on the day before evening saliva collection were unrelated to T levels in both mothers ($ps > .82$) and fathers ($ps > .24$). The hours that the parents were available for the children in a normal week were not significantly associated with T levels (mothers: $ps > .34$; fathers: $ps > .10$). T levels were also unrelated to other possible confounding variables such as working hours (mothers: $ps > .63$; fathers: $ps > .39$) and sleep quality (good, bad, reasonable) on the night before morning saliva collection (mothers: $ps > .08$; fathers: $ps > .87$). In addition, T levels were also unrelated with paternal depression ($ps > .31$), but for mothers a positive correlation was found between evening T and depression ($p < .049$). However, the associations between T and parenting quality of mothers were similar after controlling for maternal depression, excluding parental depression as potential confounding factor.

Mothers in families with a third infant child ($n = 25$) had lower diurnal variation in T ($M = 0.95$, $SD = 0.55$) than mothers in families with only two children ($M = 1.23$, $SD = 0.43$; $t(122) = 2.30$, $p < .05$). Mothers' morning and evening T levels were not different in families with and without a third child (morning T: $t(122) = 1.82$, $p = .07$; evening T: $t(122) = -0.05$, $p = .96$). Fathers' T levels were not different in families with and without a third child (morning T: $t(215) = 0.32$, $p = .75$; evening T: $t(215) = 0.00$, $p = .99$; diurnal change in T: $t(215) = 0.01$, $p = .99$). Analyses with and without families with a third child yielded similar results, so these families were retained in the current data set.

Table 1
Descriptive statistics for parents' T levels, sensitivity, and respect for autonomy.

	Mother (N = 124)	Father (N = 216)
	M (SD)	M (SD)
1. Sensitivity oldest	24.29 (2.47)	23.20 (2.69)
2. Respect for autonomy oldest	21.43 (3.03)	21.02 (2.98)
3. Sensitivity youngest	24.65 (2.73)	23.80 (2.72)
4. Respect for autonomy youngest	20.98 (3.30)	20.43 (3.07)
5. Morning T	155.13 (48.41)	342.35 (80.10)
6. Evening T	74.81 (28.32)	169.85 (44.00)
7. Diurnal change in T	1.17 (0.54)	1.10 (0.58)

Note. Means and standard deviations for T represent winsorized data.

Tables 1 and 2 display the descriptive statistics and correlations for all study variables for fathers and mothers. Consistent with the diurnal curve of testosterone, morning levels were higher than evening levels in both mothers, $t(123) = 26.04, p < .01, d = 2.03$, and fathers, $t(215) = 34.14, p < .01, d = 2.67$. Evening T and morning T were significantly correlated for both mothers, $r(124) = .71, p < .01$, and fathers, $r(216) = .36, p < .01$. As expected, fathers' T levels were significantly higher than mothers' T levels in the morning, $t(105) = 20.59, p < .01, d = 2.77$, and in the evening, $t(105) = 18.92, p < .01, d = 2.42$. There was no difference between mothers and fathers in ratio of diurnal change in T, $t(105) = -0.70, p = .48$. In couples, mothers' and fathers' T levels were unrelated for the morning assessment, $r(106) = .06, p = .55$, as well as for the evening assessment, $r(106) = .13, p = .18$, and the ratio of diurnal change in T: $r(106) = -.02, p = .86$.

Associations between T and parenting quality

For fathers, no associations were found between T levels and parenting quality ($ps > .10$). However, positive correlations were found between fathers' diurnal change in T and their sensitivity, $r(216) = .16, p = .02$, and respect for autonomy, $r(216) = .14, p = .047$, towards the youngest child, indicating that higher diurnal variation of T was associated with more optimal parenting in fathers, as can be seen in Fig. 1. For mothers, significant correlations between ratio of diurnal change in

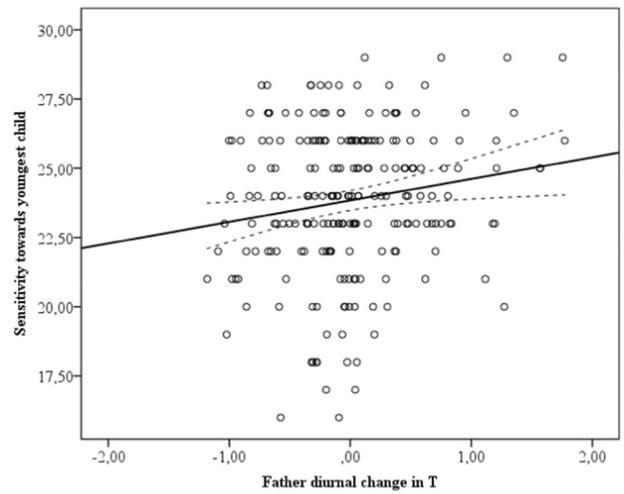


Fig. 1. Scatterplot of the association between fathers' diurnal change in T and sensitivity towards the youngest child. Note. X-axis presents T variability scores controlled for age and weight. More positive residuals represent larger variability in T during the day (i.e., increase during the night, or decrease during the day).

T and sensitivity and respect for autonomy were also found, but in the opposite direction (Fig. 2). Higher diurnal variation in T was associated with less sensitivity, $r(124) = -.25, p = .01$, and respect for autonomy, $r(124) = -.18, p = .045$, to the youngest child and less sensitivity, $r(124) = -.29, p < .01$, to the oldest child. Higher evening T levels in mothers were associated with more sensitive parenting towards the youngest, $r(124) = .18, p = .04$, and oldest child, $r(124) = .30, p < .01$. The pattern of results was the same for boys and girls, as well as for analyses controlling for parental educational level.

Multi-level analyses, taking into account that mothers and fathers are nested within couples, confirmed the results of the correlation analyses. Further, partner's T levels and partner's diurnal change in T were not significantly related to parents' own sensitivity and respect for autonomy with oldest and youngest children ($ps > .18$). No significant

Table 2
Correlations between parents' T levels, sensitivity, and respect for autonomy.

Mother	Father						
	1	2	3	4	5	6	7
1. Sensitivity oldest	.05	.41**	.28**	.16*	-.04	-.07	.03
2. Respect for autonomy oldest	.26**	.11	.05	.25**	.06	.01	.06
3. Sensitivity youngest	.37**	.20*	.10	.48**	.05	-.11	.16*
4. Respect for autonomy youngest	.16†	.41**	.56**	.26**	.08	-.11	.14*
5. Morning T	.11	.04	.00	-.04	.06	.36**	.48**
6. Evening T	.30**	.14	.18*	.08	.71**	.13	-.58**
7. Diurnal change in T	-.29**	-.16†	-.25**	-.18*	.20*	-.51**	-.02

Note. Correlations with T levels are based on residual scores for T. Correlations below the diagonal refer to associations among maternal behaviors and T levels (N = 124), correlations above the diagonal refer to associations among paternal behavior and T levels (N = 216), and correlations on the diagonal refer to associations between mothers and fathers (N = 106). Shaded area represents correlations related to the studies' hypotheses.

**p < .01, *p < .05, †p < .1.

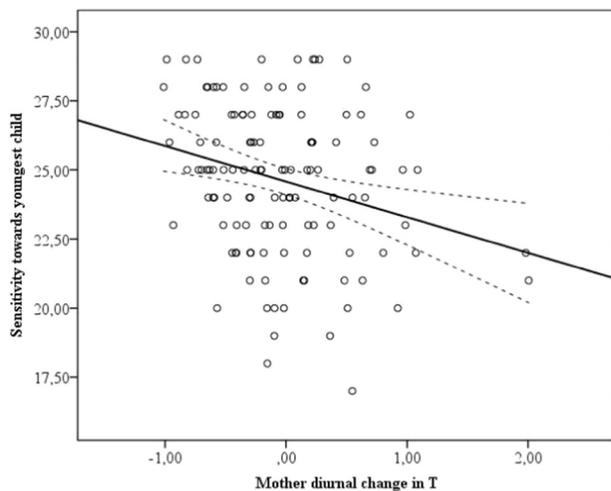


Fig. 2. Scatterplot of the association between mothers' diurnal change in T and sensitivity towards the youngest child. *Note.* X-axis presents T variability scores controlled for age and weight. More positive residuals represent larger variability in T during the day (i.e., increase during the night, or decrease during the day).

interactions between parent sex and partner's T levels or partner's diurnal change in T were found ($ps > .07$).

Discussion

To our knowledge, this is the first study that examined T and diurnal variability in T in relation to parenting quality in mothers and fathers. The results show that diurnal T variability was associated with parents' sensitivity and respect for autonomy towards their children. Interestingly, the direction of effects was different for mothers and fathers. For fathers, more diurnal variability in T was associated with *more* sensitivity and respect of autonomy to their youngest children. For mothers, more diurnal variability in T was associated with *less* sensitivity to both children and *less* respect of the youngest child's autonomy. With regard to basal T levels, higher evening T levels in mothers were associated with more sensitivity to the oldest and the youngest child.

It appears that for fathers a flexible or variable T system might be most optimal for parenting. In contrast, in mothers a more flexible T system seems to be less optimal for parenting. Our findings are consistent with a study that found that diurnal variability in T was differently associated with problem behavior in adolescent boys and girls (Granger et al., 2003), indicating that in males less diurnal variability in T might be associated with non-optimal behavior, whereas in females more diurnal variability in T might be associated with non-optimal behavior.

Our findings suggest that the T system plays a different role in mothers' and fathers' parenting behaviors. It has been suggested that alterations in testosterone levels in males reflect a shift between conflicting reproductive strategies and that these variations in testosterone levels enable men to change from mating efforts to parenting efforts (Gray and Anderson, 2010). This proposition has already been illustrated in multiple studies in more than 60 bird species that showed that testosterone levels increase when males compete for food and territory and decrease when they care for offspring (Wingfield et al., 1990). In human fathers, a similar pattern has been found. Although limited to more acute changes in T (within 40 min after a free play session), one study found that fathers who show a decrease in testosterone levels in response to interaction with their child are more likely to have a positive relationship with their child compared with fathers who show a smaller or no decrease in testosterone levels (Weisman et al., 2014). Because continuously high testosterone levels would interfere with successful parenting and continuously low testosterone levels would decrease mating success, it may thus be essential for fathers to have a

flexible testosterone system. However, it should be noted that more behavioral evidence is needed to support these speculations.

For mothers, on the other hand, the ability to lower their testosterone levels might be less necessary to achieve optimal parenting, because their testosterone levels are already substantially lower than those of fathers. A flexible testosterone system in relation to caregiving might even be less adaptive for mothers, because a certain level of testosterone seems to be necessary to respond appropriately to challenging parenting contexts (e.g., baby cries). There is evidence that the administration of testosterone in women enhances, rather than suppresses, neural responsiveness to baby cries in women (Bos et al., 2010). However, this finding could be the effect of multiple neurobiological mechanisms. For example, aromatase metabolizes testosterone to estradiol in the central nervous system, which in turn is essential for the synthesis of oxytocin (e.g., Cornil et al., 2006). Estradiol and oxytocin both promote mother–infant bonding and stimulate parental behavior (e.g., Insel and Young, 2001; Kendrick, 2000). Animal studies have shown that in mice the conversion of testosterone to estradiol stimulates parenting behavior (e.g., Trainor and Marler, 2001). Although the administration of testosterone results in an approximate 10-fold increase in blood levels of testosterone (Tuiten et al., 2000), which is not representative of general testosterone levels in women, it might be the case that a certain amount of testosterone is essential for parenting behavior.

Another explanation for the divergent role of T in mothers' and fathers' parenting behaviors may be related to differences in T synthesis and steroid systems between the sexes. While in males T is produced in large quantities primarily by the testes, in females it is synthesized in much smaller quantities in the adrenal cortex and ovaries (Miller and Tyrrell, 1995). As the adrenal cortex is highly responsive to environmental influences (e.g., stress), the production of T levels in mothers during the day is likely to reflect both physiological and environmental/social processes (Brown and Spencer, 2013). This means that females may not modulate T levels in a similar way as males do (Goymann and Wingfield, 2014). In males, the lower T levels associated with fatherhood may be the result of a down-regulation of T production by the testes (Kuzawa et al., 2009).

In addition, it is also possible that sex differences in testosterone variability in response to parenting are associated with different neural substrates in the brain. Males have been found to be more responsive than females to the behavioral and neuroendocrine actions of androgens (e.g., Fernández-Guasti et al., 2000; Roselli, 1991). Although the reasons for these differences in adult responsiveness to androgens are not fully understood, it has been suggested that structural and functional sex differences in the central nervous system play an important role. Several studies have shown that there are sex differences in the concentrations of androgen receptors (AR) in the rat brain (e.g., Simerly et al., 1990; Roselli, 1991). For example, one study showed that in at least a few brain regions in the rat brain, there appeared to be sex differences in either the number of AR cells or the relative density of labeling over certain cell groups (i.e., medial preoptic nucleus contained larger number of AR cells in males than in females, whereas the anteroventral periventricular nucleus of the preoptic area contained more AR cells in females than in males, Simerly et al., 1990). Although no absolute sex differences in the amount of AR in the rat brain were found, these sex differences in AR distribution may explain differences in the effect of T on behavior in males and females. However, more research is required to examine whether these findings also apply to humans and how these neurobiological sex differences in the T system are related to parenting behavior.

The relation between T variability and parenting quality in mothers and fathers was most profound for parental sensitivity and respect for autonomy to their youngest child. To date, only two studies examined differences in T levels among mothers in relation to child age and showed that mothers with young offspring had lower T levels in the morning than mothers with older children and non-mothers (Barrett et al., 2013; Kuzawa et al., 2010). The more frequent and demanding

activities that are required when caring for infants and young children compared with older children may contribute to the lower T levels in these mothers. This line of reasoning fits with previous studies showing that T is often related to mood disturbances among mothers with young infants (Buckwalter et al., 1999; Hohlagschwandtner et al., 2001). However, not all studies find significant links between T and mood or parental responsiveness in mothers with young infants (Fleming et al., 1997). Although no previous study examined the effect of child age on T variability in parents, we speculate that diurnal variability in T may also play a larger role in parenting young children than older children, because of the more frequent and demanding care and more close body contact with young children. This argument is supported by our finding that mothers with an infant child had lower diurnal variation in T than mothers with three- to five-year-old children.

In light of the challenge hypothesis (Wingfield et al., 1990) it is somewhat surprising that for fathers we found significant associations with T variability and not with basal T levels. We expected lower basal T levels to be associated with more optimal parenting in fathers, but this was not confirmed in our study. An explanation for the fact that basal T levels in our study were not associated with fathers' parenting quality, might have something to do with our measure of parenting. Previous research has linked lower basal T levels to fatherhood (Gettler et al., 2011b) and more optimal father-child behaviors (Mascaro et al., 2013; Weisman et al., 2014). However, in these studies the measures of parenting were often quantitative in nature (i.e., fathers versus non-fathers, time spent in childcare). Although some studies (Mascaro et al., 2013; Weisman et al., 2014) included aspects of parental involvement (i.e., frequency of gaze to infant or infant-directed vocalization, self-reported caregiving quality), we used a measure of parenting more explicitly focusing on the *quality* of parental involvement rather than only the *amount* of involvement. Lower basal T levels might be more related to quantitative aspects of fathering, whereas T variability is more closely linked to qualitative aspects of fathering. Evidence for this proposition can be found in a previous study showing that variations in a quantitative construct, relationship status (i.e., being in a committed relationship or not), was explained by basal T levels, whereas individual differences in qualitative behavioral and personality characteristics (i.e., type A personality, perfectionism, self-monitoring, sensation seeking) were explained by diurnal fluctuation in T (Sakaguchi et al., 2006).

In mothers higher evening T levels were related to more sensitive parenting, which is not in line with the challenge hypothesis or with two previous studies showing that motherhood was related to lower morning T (Barrett et al., 2013; Kuzawa et al., 2010). In both studies a quantitative aspect of mothering was assessed (i.e., being a mother or not), whereas in the current study we focused on qualitative aspects of parenting. Again, T may be differentially related to quantitative and qualitative aspects of parenting. In addition, our results indicate that the challenge hypothesis might not be applicable to females. Especially the prediction that lower T levels might facilitate parenting in mothers might not be applicable, because a previous study showed that lower T levels in adolescent girls were associated with more externalizing behavior problems (Booth et al., 2003). Clearly, more research is needed to examine whether the link between T and maternal behavior is indeed different from what would be predicted by the challenge hypothesis.

It is worth mentioning that we were able to rule out several alternative explanations for the associations between T and parenting quality in mothers and fathers. Parents' working hours, sleep quality, caregiving hours on the day before T sampling, and symptoms of depression did not confound the results. The fact that we did not find an association between caregiving hours on the day before T sampling and T levels suggests that individual differences in T levels or T variability over the day can be viewed as trait-like biological predispositions (Granger et al., 2003), explaining individual differences in parenting behavior. Apparently, T is not only suppressed in response to cues associated with marriage, children, child care, or parenting, but individual

differences in the T system might also predict individual differences in parenting behavior. Additionally, routine time spent with children was unrelated to parents' T levels (or diurnal variability in T) and parenting quality. Therefore, no evidence was found for a pathway from parental involvement, via T levels, to parenting quality, or for a pathway in which parental involvement is a mediator of the association between T and parenting quality.

There are some issues that should be taken into account when interpreting the results of this study. First, the sample consisted of predominantly highly educated Caucasian parents. Since parental role division and parenting practices are known to differ as a function of socioeconomic status and ethnicity, our findings cannot be generalized to populations from different backgrounds. Second, variability in T was based on only two saliva samples. Since T levels follow a diurnal rhythm (Booth et al., 2006; Cooke et al., 1993), a more comprehensive approach (i.e., collecting saliva in the morning, before noon, and again in the late afternoon and evening) is more optimal to capture the individual variability of T over the day. Third, the effect sizes for the statistically significant correlations between T levels and parenting in this study (Cohen's *d* between 0.32 and 0.63) are small to moderate, but similar to other predictors of sensitivity and respect for autonomy as assessed with the EAS, such as parental psychological functioning (e.g., van Ee et al., 2012; Cohen's *d* between 0.41 and 1.12) and attachment representation (e.g., Biringen et al., 2000; Cohen's *d* between 0.22 and 0.98). Last, our cross-sectional design does not allow for firm conclusions on the direction of effects. This is a pertinent problem in the literature on T and parenting. Previous studies have shown that variations in basal testosterone levels were associated with variations in parental behavior (Weisman et al., 2014), but at the same time cues associated with marriage, children, child care, or parenting can lead to short-term or longer-term fluctuations around this basal level (Gettler et al., 2011b; Kuzawa et al., 2010). Recently, Gettler et al. (2015) published the first longitudinal evidence suggesting that men who increased their involvement in caregiving during the study's period showed declines in T. However, the authors were not able to explicitly determine the direction of the initial effect and hypothesize that shifts in paternal care and T may bi-directionally influence each other (Gettler et al., 2015).

More longitudinal and experimental research is necessary to test whether T and parenting are bi-directionally related. In these studies it would also be important to take estrogen levels into account, as they might be meaningful in relation to mothers' mating and parenting behaviors. Similarly, there is a need for studies examining the interplay between T and cortisol in relation to parenting, as the HPA and HPG systems are highly interactive. For example, one study showed that mating-oriented men were more likely to have co-elevated T and cortisol levels, while parenting-oriented men were more likely to have co-downregulated T and cortisol levels (Gettler et al., 2011a). Moreover, men's T and cortisol levels have been found to decline whereas estradiol levels increase after the birth of their first child (Berg and Wynne-Edwards, 2001). In addition, a study examining hormone changes throughout the transition to parenthood demonstrated that women showed large prenatal increases in T and cortisol, whereas men showed significant declines in T and no detectable changes in cortisol (Edelstein et al., 2014). These findings underscore the need of studying the interplay between hormonal processes in both men and women in experimental designs and in longitudinal designs following the process of becoming parents.

To conclude, our results show that parenting quality in mothers and fathers is associated with diurnal variability in T. Importantly, T variability was differently associated with parenting behavior of mothers and fathers. For fathers, more diurnal variability in T was associated with more optimal parenting, whereas for mothers more diurnal variability in T was associated with less optimal parenting. These findings suggest that the T system might act differently in relation to parenting behavior in men and women, and call for further research of the processes underlying sex differences in the association between T variability and parenting behavior.

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