Reactive aggression tracks within-participant changes in women’s salivary testosterone

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The relation between testosterone and aggression has been relatively well documented in men, but it is less well understood in women. Here we assessed the relationship between salivary testosterone and reactive aggression (i.e., rejection rate for unfair offers) in the Ultimatum Game. Forty naturally cycling women were tested twice, once in the late follicular phase (around ovulation) and once during the luteal phase. Ovulation was determined using urine test strips measuring luteinizing hormone levels. Salivary samples were assayed for testosterone, estradiol, progesterone and cortisol at both test sessions. There was no association with the cycle, but multilevel modeling revealed a significant within-participant association between testosterone and rejection rate for extremely unfair offers (i.e. high reactive aggression), indicating that women showed greater reactive aggression when their testosterone levels were higher. Additionally, we found that women with relatively high individual concentrations of testosterone were more likely to reject extremely unfair offers than women with relatively low concentrations of testosterone. This study is the first to demonstrate that women react more aggressively in response to provocation when their testosterone level is high than when their testosterone is low, suggesting that testosterone plays an important role in the regulation of women’s aggressive behavior following social provocation.
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Reactive aggression tracks within-participant changes in women’s salivary testosterone

In non-human animals testosterone (T) plays a key role in status seeking behavior such as aggression and competition (McCall & Singer, 2012) and many researchers suggest a similar effect of testosterone in humans (Carré, McCormick, & Hariri, 2011; Eisenegger, Haushofer, & Fehr, 2011; Mazur & Booth, 1998). Indeed, human endogenous testosterone levels have been shown to correlate positively with dominance-seeking behavior (Archer, 2006; Mazur & Booth, 1998) and to increase vigilance for status threats (van Honk et al., 1999). Yet, the relationship between individual differences in testosterone and aggressive behavior is relatively weak (Archer, Graham-Kevan, & Davies, 2005) and is moderated by biological sex and social context (Carré & Olmstead, 2015; Josephs, Mehta, & Carré, 2011).

Aggressive behavior often occurs as a retaliatory response to social provocation (Bettencourt, Talley, Benjamin, & Valentine, 2006). Several studies have examined the relationship between endogenous baseline testosterone concentrations and aggressive behavioral reactions to social provocation as measured with the Ultimatum Game (Burnham, 2007; Guth, Schmittberger, & Schwarze, 1982; Güth, Schmittberger, & Schwarze, 1982; Mehta & Beer, 2010). In this game, two anonymous individuals, a proposer and a responder, have to agree on how to split a certain amount of money. The proposer makes an offer (ultimatum) and the responder can either accept the offer, or reject it. If the responder accepts the offer, the money is divided as suggested by the proposer. If the responder decides to reject the offer, neither of the two receives anything. Responders who are motivated purely by self-interest should accept all offers. Accepting every offer would correspond to what would be expected from an economic point of view. Contrary to this prediction, several studies have shown that responders do not maximize monetary profit but tend to reject offers below 20% of the stake (Camerer & Thaler, 1995), even when there will be no future interactions with the partner (Güth et al., 1982). According to several authors (Carré et al., 2011; Crockett, Clark,
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Tabibnia, Lieberman, & Robbins, 2008; Mehta & Beer, 2010), rejecting an unfair offer is a form of reactive aggression as it is committed with the intent to cause financial harm to the selfish proposer even at personal cost (costly punishment). Rejection of unfair offers has thus been interpreted as a form of non-physical aggression in response to social provocation and status threat (Cuev et al., 2017; Yamagishi et al., 2012).

If testosterone modulates aggressive behavior, it is plausible to assume that rejections of unfair offers in the Ultimatum Game are positively related to testosterone levels. Indeed, two studies reported a positive relationship between endogenous testosterone levels and rejection rates of unfair offers in men (Burnham, 2007) as well as in mixed-sex samples (Mehta & Beer, 2010; but see Mehta, Mor, Yap, & Prasad, 2015). However, these findings could not be replicated in a sample consisting only of women (Eisenegger, Naef, Snozzi, Heinrichs, & Fehr, 2010). Similarly, testosterone administration is associated with high rejection rates in men (Zak et al., 2009; but see Kopsida, Berrebi, Petrovic, & Ingvar, 2016) but not in women (Eisenegger et al., 2010; Kopsida, Berrebi, Petrovic, & Ingvar, 2016; Zethraeus et al., 2009). In several other previous studies it has been found that T-aggression relations are stronger and more consistent for men than for women (Carré, Campbell, Lozoya, Goetz, & Welker, 2013; Carré, Putnam, & McCormick, 2009; Gladue, 1991; Inoff-Germain et al., 1988; Susman et al., 1987).

Several speculations can be made to explain the weak relationship between testosterone and aggressive behavior in women: First, testosterone might not affect women in the same way as it affects men (see Carré et al., 2011; Carré & Olmstead, 2015). Second, the influence of hormones and social factors may differ between sexes: Women may be better able than men to inhibit their behavioral responses (Inoff-Germain et al., 1988; for a meta-analysis see Cross, Copping, & Campbell, 2011). Third, some researchers have suggested that the effects of testosterone on aggressive behavior are dependent on the gonadal hormone's conversion to estradiol through the enzyme aromatase (Trainor & Nelson, 2012). Indeed,
there is some evidence that estradiol may be related to aggressive behavior in women (Brambilla, Speca, Pacchiarotti, & Biondi, 2010; Finkelstein et al., 1998).

In the majority of studies with female participants the following two considerations, which could potentially have confounding effects on T-behavior associations, have been neglected: Menstrual cycle phase and use of hormonal contraception. Estradiol, progesterone and testosterone levels vary strongly as a function of cycle phase (Sherman & Korenman, 1975) and hormonal contraceptives artificially suppress endogenous sex hormone levels throughout the menstrual cycle (Basu et al., 1992; Rivera, Yacobson, & Grimes, 1999; van Heusden & Fauser, 2002). Collapsing over hormonally distinct cycle phases and/or over women who do and do not use hormonal contraceptives may result in distorted results.

We aimed to re-assess the relationship between testosterone and reactive aggression in naturally cycling women. Each woman was tested at two specific time points of her menstrual cycle, enabling us to examine whether aggressive behavior is modulated by menstrual cycle phase as a result of changing sex hormone levels (e.g. Sherman & Korenman, 1975).

To date, evidence for changes in aggressive behavior across the menstrual cycle is mixed. Some studies report an increase in women’s aggression during late luteal phase (D'Orban & Dalton, 1980; Dalton, 1961; Ritter, 2003) while others found no effect of menstrual cycle on aggressive behavior in healthy participants (Bond, Critchlow, & Wingrove, 2003; Brambilla et al., 2010; Dougherty, Bjork, Cherek, Moeller, & Huang, 1998; Dougherty, Bjork, Moeller, & Swann, 1997). However, some evidence suggested that women become more intra-sexually competitive in the late follicular phase (fertile window) of the menstrual cycle (Eisenbruch & Roney, 2016; Fisher, 2004; Lucas & Koff, 2013; Lucas, Koff, & Skeath, 2007). Specifically, researchers found that women in the fertile window are more likely to reject unfair offers of other women in the Ultimatum Game (Eisenbruch & Roney, 2016; Lucas et al., 2007).
Due to several methodological limitations these findings should be interpreted with caution. First, some studies relied on self-report measures of aggression (Bond et al., 2003; Brambilla et al., 2010; Ritter, 2003), potentially resulting in biased assessments of aggressive behavior (Carré & Olmstead, 2015). Second, most studies determined the menstrual cycle phase by participant’s self-reported cycle dates (e.g., retrospectively recalled date of last menstrual onset), instead of determining ovulation by means of ovulation tests (Brambilla et al., 2010; Dalton, 1961; Eisenbruch & Roney, 2016; Lucas & Koff, 2013; Lucas et al., 2007; Ritter, 2003). Self-reported cycle dates are prone to measurement errors and do not allow detecting anovulatory cycles (Fehring, Schneider, & Raviele, 2006; Gangestad et al., 2016; Small, Manatunga, & Marcus, 2007). Finally, most studies did not collect hormonal data (Dalton, 1961; Dougherty et al., 1998; Eisenbruch & Roney, 2016; Lucas & Koff, 2013; Lucas et al., 2007; Ritter, 2003), thus limiting the conclusion about hormonal effects on aggressive behavior. In summary, no study to date has provided a definitive answer to whether or not aggressive behavior is modulated by cyclic changes in endogenous hormone levels.

In the present study, we set out to test (i) whether reactive aggression changes across the menstrual cycle and whether such an effect depends on the sex of the aggressor, (ii) whether within-participants’ change in testosterone correlates with changes in reactive aggression and (iii) whether high individual testosterone levels (between-participants) are associated with high reactive aggression in naturally cycling women. Our analyses also considered potential effects of estradiol, progesterone and cortisol on aggressive behavior and possible moderation effects of cortisol on the relationship between testosterone and aggressive behavior. This was undertaken because previous studies have suggested a modulating effect of cortisol on the testosterone-aggression relationship (Denson, Mehta, & Tan, 2013; Mehta et al., 2015; Mehta & Prasad, 2015; Popma et al., 2007). We employed a within-participant design in which women acted as responders in the Ultimatum Game, once during the late follicular phase (around ovulation) and once during the luteal phase. During
both test sessions, participants provided a saliva sample from which levels of testosterone, estradiol, progesterone, and cortisol were assessed. Multilevel modeling was then used to test whether changes in rejection rate of unfair offers were predicted by changes in salivary testosterone. The advantage of using multilevel regressions is that we can enter participants as level-2 variable with lab sessions (hormones and rejection rates) nested within participants, enabling us to analyse the data with respect to within-participant and between-participant variation without aggregating scores. We predicted that (a) within-participant higher testosterone levels predict a higher rejection rate of unfair offers and (b) differences in testosterone between women show a similar pattern like within women in that women with higher testosterone levels show increased rejection rates of unfair offers.

Methods

Participants

Initially, 68 women showed interest in taking part in this study. In the end, 40 women ranging in age between 19 and 38 years ($M = 25.91$, $SD = 5$) completed the study (see flow chart in Supplementary Online Material for an overview of the participants who dropped out and the reasons for non-participation at each stage). Participants were financially compensated based on their decisions in the Ultimatum Game ($M = $50.55, $SD = $8.20; $CHF 1 = $1.05). All provided written informed consent to take part in this study. The study protocol was approved by the ethics committee of the Faculty of Human Sciences of the University of Bern and was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Session scheduling

All participants were initially screened in a telephone interview. Only women who reported having regular menstrual cycles (i.e., between 25 and 35 days in length and no more
than 5 days variation between cycles) as well as fulfilling the following criteria were eligible to take part in the study: i) no use of hormonal contraception for at least the three previous months, ii) no suspected or confirmed pregnancy, ii) no breastfeeding, iv) no use of any kind of medication, and v) aged between 18 and 40 years. Cycle information provided in the telephone interview was used to facilitate ovulation determination (see below).

Each participant came into the laboratory twice. One experimental session took place during late follicular phase (around ovulation) and the other during the luteal phase. The order of the test sessions was counter-balanced across participants and both test sessions took place at the same time of day. This was done to control for diurnal changes in hormone levels. Ovulation was determined by means of urine samples using One Step Ovulation Test strips. These ovulation test strips measure the metabolite concentration of the luteinizing hormone (LH) in urine. The surge in LH provokes ovulation in the upcoming 24-36 hours. Four days prior to expected ovulation women started using ovulation test strips twice a day (morning and evening). Expected ovulation was based on the backward counting method. Participants photographed each test using their smart phones and sent the picture to the study manager, who verified whether the test was positive or not. After positive testing, the women immediately reported to the laboratory and were then either tested within 48 hours of LH surge and then again 7 days later (late follicular-luteal group) or they were scheduled 7 days after the measured peak of the LH surge (luteal-late follicular group). Participants assigned to the luteal-late follicular group again assessed the LH surge in the following cycle and were then tested within 48 hours of the next LH peak.

**Ultimatum Game**

In each session, every participant received 24 offers of 24 different anonymous proposers (12 male and 12 female) on a hand-written slip of paper (the full experimental instructions are provided in the Supplementary Material). The sex of proposer was
represented by male and female names and by different colors of the offer (i.e. pink for female proposer and blue for male proposer). Participants were told that the proposers were students of another University who had submitted their offers beforehand and that they were not present. In reality each participant received the same 24 offers of virtual proposers. Because a recent study showed that stake size influences the responder’s behavior (Andersen, Ertac, Gneezy, Hoffman, & List, 2011) we included two stake sizes: CHF 24 and CHF 6. All offers fell into one of three fairness categories: fair (50% of stake), slightly unfair (30% of stake), or extremely unfair (16.7% of stake). The total number of offers consisted of 8 fair offers, of which 4 were high stake offers (12 CHF of 24) and four were low stake offers (3 CHF of 6); 8 slightly unfair offers of which 4 were high stake offers (8 CHF of 24) and four were low stake offers (2 CHF of 6); 8 extremely unfair offers of which 4 were high stake offers (4 CHF of 24) and 4 were low stake offers (1 CHF of 6). The offers were presented in random order. Participants received identical offers at each session, but the proposers were represented by different names and the order of offers was randomized to disguise this. Previous studies that have used simulated players often assume (or at least do not report whether) participants believed the cover story. In the present study, at the end of the second test session participants filled out a questionnaire to check whether they had suspected that the offers were not real. Importantly, 90% of the participants believed that the interactions were real and with actual players. Before leaving the laboratory, participants were fully debriefed and they tossed a coin to select whether the earnings of Session 1 or Session 2 were paid (all accepted offers in the selected session were paid).

**Salivary analysis**

Each participant provided saliva samples at the beginning of each session from which steroid hormone levels (testosterone, estradiol, progesterone, and cortisol) were determined. Participants were instructed to refrain from eating and to abstain from caffeine and smoking for at least 1h prior each experimental session. Participants were asked to rinse their mouth
with fresh water and to wait approximately 5 min before providing saliva. Samples were collected using a commercially available sampling device (SaliCaps, IBL, International, Hamburg, Germany). The saliva samples were stored at -28°C and were later analyzed by an independent laboratory (Dresden Lab Service GmbH, Dresden, Germany) using commercially available radioimmunoassay kits adopted for the analysis of salivary hormones levels (IBL International, Hamburg, Germany). The average intra- and inter-assay variance for testosterone, estradiol, progesterone and cortisol were all below 11%.

**Statistical analysis**

Statistical analyses were performed using SPSS 23.0 and level of significance was set at $p < .05$. Examination of hormonal data revealed that the distributions were positively skewed. Therefore, log transformations were used to achieve normal distributions prior to analyses. The outlier-labeling rule (Hoaglin, Iglewicz, & Tukey, 1986) was applied to define outliers in testosterone, estradiol, progesterone and cortisol levels. This rule declares observations that lie more than 2.2 times the interquartile range away from the nearest quartile as outliers and is resistant to extreme values. For each hormone, outliers were winsorized (Dixon, 1960). All analyses used the log-transformed hormonal values; however, raw data are reported in Table 1 to facilitate comparison with prior studies. $T$-tests for dependent samples were used to compare hormonal levels between the two cycle phases. We tested for within-participant effects and between-participant effects of salivary testosterone on rejection rate using multilevel modeling. Specifically, to test our three research questions we used binary logistic multilevel regressions for repeated measurements. To test for within-participants effects of testosterone on rejection rates for each unfair offer type, rejection rates were used as the dependent variable and values for testosterone were entered as predictors, at the test session level (Level 1). The rejection rate was the dependent variable and was binary-coded (0 = accept, 1 = reject). For our purposes, we analyzed only unfair offers because we expected
to find a relation between testosterone and reactive behavior only in situations where social provocation is present (i.e. unfair offers). The multilevel null-model (i.e., without predictors) revealed a significant proportion of inter-individual variance in rejection rate, \( s^2(\beta_{0t}) = 2.329, z = 3.351, SE = 0.695, p = .001 \). Pseudo \( R^2 \) of inter-participant variance was .08 legitimating a multilevel regression analysis (Snijders & Bosker, 2004).

**Coding and centering of all predictor variables for multilevel analyses:** In the following analyses we included three dichotomous predictors on Level 1: *Fairness* (0 = slightly unfair offers, 1 = extremely unfair offers), *Stake size* (0 = low stake size, 1 = high stake size), and *Cycle phase* (0 = late follicular phase, 1 = luteal phase). All hormone levels (*Testosterone, Estradiol, Progesterone*, and *Cortisol*) were grand mean-centered across the entire sample. Thus, the value 0 represents the average hormone level of the sample.

**Multilevel models – Research Question 1. Does rejection rate change across the menstrual cycle?** We investigated whether rejection rate changed across the menstrual cycle and was influenced by the experimental variables *Fairness* and *Stake size*. Thus, the first model included *Fairness* \((F_{it})\), *Stake size* \((S_{it})\), *Cycle phase* \((CP_{it})\), and all interactions between these variables as within participants (Level 1) predictors of rejection rate (see Supplemental Material, Equation 1a).

According to previous research findings (Eisenbruch & Roney, 2016; Fisher, 2004; Lucas & Koff, 2013; Lucas et al., 2007), which suggested higher intra-sexual competition during late follicular phase, we also investigated whether the *Proposer sex* \((PS_{it}, 0 = \text{male proposer}, 1 = \text{female proposer})\) modulates the relationship between cycle phase and rejection rate and the relationship between fairness and cycle phase (see Supplemental Material, Equation 1b).

**Multilevel models – Research Question 2. Does within-participant testosterone level predict variance in rejection rate?** We investigated whether the experimental variables *Fairness* and *Stake size* as well as within-participants change in *Testosterone* is associated
with rejection rate. In a first step, Fairness ($F_{it}$), Stake size ($S_{it}$), and Testosterone ($T_{it}$) and all interaction terms between these variables (Level 1) were entered in the model (see Supplemental Material, Equation 2a). In a second step, these effects were controlled for Estradiol ($E_{it}$), Progesterone ($P_{it}$), and Cortisol ($C_{it}$, (see Supplemental Material, Equation 2b).

It is conceivable that the effect of testosterone is modulated by cortisol (Mehta & Prasad, 2015; Popma et al., 2007). Therefore, we additionally included all possible interaction terms with Cortisol ($C_{it}$) resulting in Equation 2c. Fairness ($F_{it}$), Stake size ($S_{it}$) and hormones, were treated as within-participant (Level 1) predictors of rejection behavior (see Supplemental Material, Equation 2c).

Multilevel models – Research Question 3. Does between-women testosterone level predict between-women variance in rejection rate? To investigate the influence of person-specific testosterone level on rejection behavior, we included individual testosterone level (mean concentration of testosterone across the two cycle phases) in the model, in addition to the experimental variables (Fairness, Stake size). Thus, in this model Testosterone ($\bar{T}_t$) was entered as between participants (Level 2) predictor. Similarly as described above, in a first step, the mean level of Testosterone ($\bar{T}_t$) was entered in the model and to control for diurnal rhythms in hormone concentrations, we additionally entered the variable Time of day ($Time_t$; [s]) at Level 2 (see Supplemental Material, Equation 3a). In a second step (see Supplemental Material, Equation 3b), all hormones were entered simultaneously at Level 2 to control for the effect of Estradiol ($E_t$), Progesterone ($P_t$), and Cortisol ($C_t$).

As mentioned in Research Question 2, the effect of testosterone might be moderated by cortisol (Mehta & Prasad, 2015; Popma et al., 2007). Therefore, we additionally included all possible interaction with Cortisol (see Supplemental Material, Equation 3c).
Results

Hormone analyses

Two participants provided insufficient saliva for assessing testosterone and were therefore omitted from the respective analyses. Hormone assessments revealed that progesterone levels were significantly higher during the luteal phase than during late follicular phase, $t(37) = -5.000, p < .001$. Cortisol levels were lower during the luteal phase than during late follicular phase, $t(37) = 2.449, p = .019$. However, levels of estradiol, $t(37) = 1.156, p = .255$, and testosterone, $t(37) = .099, p = .922$, did not differ between the two phases. Average hormone levels during each cycle phase are shown in Table 1.

Research Question 1: Does reactive aggression change across the menstrual cycle?

Consistent with previous studies using the Ultimatum Game (Güth et al., 1982; Sanfey, Rilling, Aronson, Nystrom, & Cohen, 2003), there was a significant difference in rejection rate between extremely and slightly unfair offers, $\alpha_{10} = 4.032, z = 5.456, SE = 0.739, p < .001$, confirming that participants were more likely to reject extremely unfair than slightly unfair offers. There was also a significant difference in rejection rate between low stake and high stake offers, $\alpha_{20} = -1.656, z = -3.570, SE = 0.464, p = .001$, revealing that low stake offers were more likely to be rejected than high stake offers. The interaction between Fairness x Stake size did not reach significance, $\alpha_{4} = 1.240, z = 1.896, SE = 0.654, p = .059$. There was no effect of Cycle phase (see Supplemental Material for more details).

Additional analyses including Proposer sex in the model revealed no significant interaction effects between Proposer sex and Cycle phase, $\alpha_{10} = -0.268, z = -0.543, SE = 0.494, p = .587$, or Fairness x Cycle phase x Proposer sex, $\alpha_{12} = 0.671, z = 1.230, SE = 0.545, p = .219$, Stake size x Cycle phase x Proposer, $\alpha_{13} = 0.760, z = 0.883, SE = 0.861, p$
= .377, or Fairness x Stake size x Cycle phase x Proposer sex, $\alpha_{14} = 0.183, z = 0.169, SE = 1.082, p = .866.$

Research Question 2: Does within-participant testosterone level predict variance in rejection rate?

We found significant effects of Fairness, $\alpha_{10} = 3.865, z = 7.086, SE = 0.545, p < .001,$ and Stake size, $\alpha_{20} = -1.903, z = -4.046, SE = 0.470, p < .001.$ Additionally, there was a significant interaction between Fairness and Stake size, $\alpha_4 = 1.421, z = 2.739, SE = 0.519, p = .007$ (see Table 2). This interaction indicates that the difference between the rejection rate for slightly unfair and extremely unfair offers is most pronounced for high stake offers. Most importantly, there was a significant Fairness x Testosterone interaction, $\alpha_5 = 2.209, z = 3.993, SE = 0.553, p < .001,$ indicating that women rejected more extremely unfair than slightly unfair offers in test sessions where testosterone was high (see Figure 1). This pattern of results was not altered when levels of Estradiol, Progesterone, and Cortisol were controlled (see Supplemental Material for more details).

Since previous work has suggested that the effect of testosterone on aggression in humans is moderated by cortisol (Mehta & Prasad, 2015; Popma et al., 2007), we ran an additional model. There was a significant Testosterone x Cortisol interaction, $\alpha_{14} = 1.45, z = 2.35, SE = 0.62, p = .019,$ suggesting that the relation between testosterone and rejection rate is modulated by cortisol: The higher the Cortisol level the stronger is the relationship between Testosterone and rejection rate. Importantly, the interaction between Fairness x Testosterone still remained significant, $\alpha_8 = 2.72, z = 2.99, SE = 0.91, p = .003$ (see Supplemental Material for more details).
Research question 3: Does between-women testosterone level predict between-women variance in rejection rate?

We found the same pattern of effects for the experimentally manipulated variables (Fairness, Stake size, Fairness x Stake size) on the mean level of testosterone (see Table 3). Furthermore, there was a significant interaction between Fairness x Testosterone, $\alpha_{11} = 5.102, z = 2.605, SE = 1.959, p = .014$, indicating that participants with generally higher levels of testosterone showed higher rejection rates for extremely unfair compared to slightly unfair offers than participants with lower testosterone levels (see Figure 2). Additionally, there was a significant Stake size x Testosterone interaction, $\alpha_{21} = 3.619, z = 2.384, SE = 1.518, p = .022$, indicating that the effect of Stake size was alleviated by testosterone levels. Specially, participants with generally high levels of testosterone showed higher rejection rates than participants with low testosterone levels, independent of the stake size. Both interactions remain significant when Estradiol, Progesterone, and Cortisol were entered in the model (see Supplemental Material for more details).

When we included Cortisol in the model, we again found a significant interaction between Fairness x Testosterone, $\alpha_1 = 5.531, z = 2.773, SE = 1.994, p = .012$. However, the Stake Size x Testosterone interaction was no longer significant, $\alpha_{21} = 2.887, z = 1.867, SE = 1.547, p = .072$. There was a significant Fairness x Stake Size x Testosterone x Cortisol interaction, $\alpha_{33} = -15.107, z = -2.629, SE = 5.746, p = .022$, indicating that rejection rate on extremely unfair offers with low stake size was influenced by testosterone levels only and not by cortisol levels. However, when stake size was high, cortisol level attenuated the testosterone effect on rejection rates of extremely unfair offers (see Supplemental Material for more details).
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Discussion

Numerous studies have demonstrated an association between basal levels of testosterone (T) and aggression in men (see Carré & Olmstead, 2015 for a review). In comparison, far fewer studies have investigated the possibility of a similar relationship between aggression and testosterone in women. The present study investigated the association between basal levels of testosterone and reactive aggression in naturally cycling women. Using a counterbalanced within-participants design, women were tested during the late follicular and luteal menstrual cycle phases. Reactive aggression was measured using a laboratory-based bargaining game (Ultimatum Game, UG). Specifically, we examined whether (i) reactive aggression (as measured by rejection rates of unfair offers) changes across the menstrual cycle, (ii) within-individual variation in testosterone levels is associated with within-individual variation in reactive aggression, and (iii) generally higher testosterone predicts reactive aggression. This study is the first to directly link within-individual changes and between-individual differences in aggression to changes in salivary testosterone levels.

We found that women showed more reactive aggression in response to extremely unfair offers in the UG in test sessions where testosterone was high. This within-individual effect of testosterone on reactive aggression for extremely unfair offers was independent of possible effects of estradiol or progesterone, none of which significantly influenced reactive aggression. Furthermore, similar to previous studies (Burnham, 2007; Mehta & Beer, 2010), we found that women with generally high testosterone levels (averaged over both test sessions) showed a higher reactive aggression behavior in response to extremely unfair offers than women with low testosterone levels. Furthermore, women with generally high testosterone levels showed higher reactive aggression independent of the stake size. Taken together, these results suggest that reactive aggression varies as a function of women’s testosterone levels.
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To date only few studies have investigated testosterone-behavior associations in women (Carré et al., 2011; Carré & Olmstead, 2015; Geniole, MacDonell, & McCormick, in press; Josephs et al., 2011). One of the difficulties in studying testosterone variation in women is that testosterone levels vary across the menstrual cycle (Dabbs & Delarue, 1991; Sherman & Korenman, 1975) and are modulated by hormonal contraception use (Basu et al., 1992; Rivera et al., 1999; van Heusden & Fauser, 2002). The present findings, using a methodologically rigorous design, help to explain inconsistent results of previous studies. The seemingly contradictory results of previous studies may have originated in not controlling for menstrual cycle phase (Carré, Baird-Rowe, & Hariri, 2014; Carré et al., 2013; Mehta & Beer, 2010; Mehta et al., 2015) and in not differentiating between normally cycling women and women using hormonal contraception (Carré et al., 2013; Mehta & Beer, 2010; Mehta et al., 2015). Our findings suggest that the ambiguous findings from previous studies derive from measurement errors, such as neglected effects of menstrual cycle and use of hormonal contraception, rather than from sex differences in aggression (Dabbs & Hargrove, 1997) or self-control (Inoff-Germain et al., 1988).

Interestingly, the T-aggression association was only found for extremely unfair offers. Rejecting extremely unfair offers causes more harm to the proposer (i.e. proposer loses more money) while the personal cost for the responder is low (i.e. she loses less money). Therefore, rejecting an extremely unfair offer is a more extreme form of reactive aggression than rejecting a slightly unfair offer. This is in line with Burnham (2007) and Zak et al. (2009), who only presented extremely unfair offers and also found a significant relationship between testosterone and rejection behavior.

Several authors suggested that testosterone and cortisol jointly regulate aggressive behavior (Carré & Mehta, 2011; Denson et al., 2013; Mehta & Prasad, 2015; Terburg, Morgan, & van Honk, 2009). In the present study, cortisol moderated within-individual effects of testosterone on reactive aggression, such that women reacted more aggressively to
unfair offers in test sessions where testosterone and cortisol levels were high. This pattern of results described here is consistent with the dual-hormone hypothesis, suggesting that testosterone predicts status-seeking behavior, such as aggressive behavior, especially when cortisol levels are high (cf. Denson et al, 2013; but see Mehta & Prasad, 2015; Popma et al., 2007).

We found no evidence for cycle-dependent changes in reactive aggression. Our results are contrary to findings reporting an increase in aggression during the late luteal phase (D'Orban & Dalton, 1980; Dalton, 1961; Ritter, 2003). Instead, our findings add to the body of literature that finds no evidence for a cyclic shift in aggression in healthy women (Bond et al., 2003; Brambilla et al., 2010; Dougherty et al., 1998; Dougherty et al., 1997). Some studies showed that women become more competitive against other women during the fertile window of the menstrual cycle (Eisenbruch & Roney, 2016; Fisher, 2004; Lucas & Koff, 2013; Lucas et al., 2007). However, we found no evidence for such an effect. A possible explanation is that the representation of proposer sex was too subtle to influence participants’ decisions. However, we note that we maximized the chance of detecting cyclic changes, if any were present, in several ways. First, we used behavioral measures instead of self-reported measures of aggression. Several studies (Bond et al., 2003; Brambilla et al., 2010; D'Orban & Dalton, 1980; Dalton, 1961; Ritter, 2003) measured aggression by self-report, such as with the Aggression Questionnaire (Buss & Perry, 1992) or the Buss-Durkee Rating Scale (Buss & Durkee, 1957). Behavioral measures of aggression are not only more valid but they are also more sensitive to state variations, such as cyclic shifts (see Archer et al., 2005; Carré & Olmstead, 2015 for review). Second, we determined the relevant menstrual cycle phases as accurately as possible using ovulation test strips and hormone assays. Most previous studies were based on self-reported menstrual cycle data (Bond et al., 2003; D'Orban & Dalton, 1980; Dalton, 1961; Eisenbruch & Roney, 2016; Lucas & Koff, 2013; Lucas et al., 2007; Ritter, 2003), which are a less valid method because participants may inaccurately recall the details.
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of the menstrual cycle (Gangestad et al., 2016; Small et al., 2007). Moreover, self-reported cycle data are prone to measurement errors because of the high intra- and inter-variability of menstrual cycle length (Fehring et al., 2006). To our knowledge, ovulation was determined with ovulation tests and sex hormone measures in only one menstrual cycle study (Dougherty et al., 1997), and in this study no cyclic changes in reactive aggression were observed. Finally, we used a within-participants design rather than a between-design to optimize the detection of small intra-individual variations in women’s behavior. Taken together, our careful cycle monitoring procedure and the sensitive measure of aggression should have increased the likelihood of finding cyclic shifts in female reactive aggression, but despite this none were evident.

Our study has some limitations that should be noted. First, we had a relatively small sample size. We note, however, that our sample size (N = 40) was larger than that of most previous studies (e.g., Bond et al., 2003: N=22; Brambilla et al., 2010: N=15; Dougherty et al., 1998: N=15; Dougherty et al., 1997: N=11; Ritter, 2003: N=29), except for one older study (Dalton, 1961 N = 386, but see methodological problems; Harry & Balcer, 1987). Second, we measured only baseline testosterone and not reactive T. According to some researchers, reactive testosterone more adequately predicts social behavior than baseline testosterone (e.g., Carré et al., 2011; Carré & Olmstead, 2015; McGlothlin, Jawor, & Ketterson, 2007). The fact that we used baseline testosterone levels might explain why we found only a weak relationship between testosterone and reactive aggression. For future research it will be an important next step to examine whether within-participant variation in reactive testosterone is associated with behavioral variation within women.

To conclude, we demonstrate that women became more aggressive in response to provocation when their testosterone level was high. Furthermore, women with generally higher testosterone levels react more aggressively in response to provocation than women
with low testosterone levels. Using a methodologically rigorous design we could thus demonstrate that testosterone plays a role in the regulation of women’s aggressive behavior.
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References


### Table 1: Hormone levels in the two menstrual phases

<table>
<thead>
<tr>
<th></th>
<th>Estradiol [pg/ml]</th>
<th>Progesterone [pg/ml]</th>
<th>Testosterone [pg/ml]</th>
<th>Cortisol [nmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Late follicular, mean (SD)</strong></td>
<td>7.38 (6.22)</td>
<td>121.98 (138.05)</td>
<td>38.42 (28.45)</td>
<td>9.81 (8.05)</td>
</tr>
<tr>
<td><strong>Luteal phase, mean (SD)</strong></td>
<td>5.93 (1.64)</td>
<td>258.18 (194.89)</td>
<td>37.41 (28.33)</td>
<td>6.50 (4.92)</td>
</tr>
<tr>
<td><strong>Paired t-test</strong></td>
<td>$p = .255$</td>
<td>$p &lt; .001$</td>
<td>$p = .922$</td>
<td>$p &lt; .050$</td>
</tr>
</tbody>
</table>

Note: paired t-tests on log-transformed hormone values appear in the table. Degrees of freedom= 35.
Table 2:

Fixed Effects:

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-1.66</td>
<td>0.49</td>
<td>-3.39</td>
<td>.002**</td>
</tr>
<tr>
<td>Fairness</td>
<td>3.87</td>
<td>0.55</td>
<td>7.09</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Stake size</td>
<td>-1.90</td>
<td>0.47</td>
<td>-4.05</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-0.329</td>
<td>0.69</td>
<td>-0.48</td>
<td>.632</td>
</tr>
<tr>
<td>Fairness*Stake size</td>
<td>1.42</td>
<td>0.52</td>
<td>2.74</td>
<td>.007**</td>
</tr>
<tr>
<td>Fairness*Testosterone</td>
<td>2.21</td>
<td>0.55</td>
<td>3.99</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Stake size*Testosterone</td>
<td>0.67</td>
<td>0.54</td>
<td>1.24</td>
<td>.218</td>
</tr>
<tr>
<td>Fairness<em>Stake size</em>Testosterone</td>
<td>-0.65</td>
<td>0.84</td>
<td>-0.78</td>
<td>.438</td>
</tr>
</tbody>
</table>

Random Effects:

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s^2(\nu_0)$</td>
<td>7.67</td>
<td>2.55</td>
<td>3.01</td>
<td>.003**</td>
</tr>
<tr>
<td>$s^2(\nu_1)$</td>
<td>4.74</td>
<td>2.21</td>
<td>2.14</td>
<td>.032*</td>
</tr>
<tr>
<td>$s^2(\nu_2)$</td>
<td>2.27</td>
<td>1.08</td>
<td>2.10</td>
<td>.036*</td>
</tr>
<tr>
<td>$s(\nu_0, \nu_1)$</td>
<td>1.13</td>
<td>1.66</td>
<td>0.68</td>
<td>.494</td>
</tr>
<tr>
<td>$s(\nu_0, \nu_2)$</td>
<td>-2.28</td>
<td>1.55</td>
<td>-1.47</td>
<td>.142</td>
</tr>
<tr>
<td>$s(\nu_1, \nu_2)$</td>
<td>0.73</td>
<td>1.73</td>
<td>0.42</td>
<td>.675</td>
</tr>
</tbody>
</table>

Significance: *** p<.001, ** p<.01, * p<.05
### Table 3:

**Fixed Effects:**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-1.65</td>
<td>0.51</td>
<td>-3.23</td>
<td>.003 **</td>
</tr>
<tr>
<td>Fairness</td>
<td>3.49</td>
<td>0.53</td>
<td>6.565</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>Stake size</td>
<td>-2.38</td>
<td>0.45</td>
<td>-5.23</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.75</td>
<td>1.84</td>
<td>0.41</td>
<td>.689</td>
</tr>
<tr>
<td>Time</td>
<td>-0.01</td>
<td>0.01</td>
<td>-1.37</td>
<td>.202</td>
</tr>
<tr>
<td>Fairness*Stake size</td>
<td>1.84</td>
<td>0.49</td>
<td>3.75</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>Fairness*Testosterone</td>
<td>5.10</td>
<td>1.96</td>
<td>2.61</td>
<td>.014 *</td>
</tr>
<tr>
<td>Stake size*Testosterone</td>
<td>3.62</td>
<td>1.52</td>
<td>2.38</td>
<td>.022 *</td>
</tr>
<tr>
<td>Fairness<em>Stake size</em>Testosterone</td>
<td>-2.47</td>
<td>1.55</td>
<td>-1.58</td>
<td>.119</td>
</tr>
</tbody>
</table>

**Random Effects:**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s^2(\nu_0)$</td>
<td>7.82</td>
<td>2.71</td>
<td>2.89</td>
<td>.004 **</td>
</tr>
<tr>
<td>$s^2(\nu_1)$</td>
<td>5.24</td>
<td>2.39</td>
<td>2.20</td>
<td>.028 *</td>
</tr>
<tr>
<td>$s^2(\nu_2)$</td>
<td>1.82</td>
<td>0.92</td>
<td>1.98</td>
<td>.048 *</td>
</tr>
</tbody>
</table>

Significance: *** p<.001, ** p<.01, * p<.05