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Raised salivary testosterone in women is associated with increased attraction to masculine faces

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Abstract

Women's preferences for masculinity in men's faces, voices and behavioral displays change during the menstrual cycle and are strongest around ovulation. While previous findings suggest that change in progesterone level is an important hormonal mechanism for such variation, it is likely that changes in the levels of other hormones will also contribute to cyclic variation in masculinity preferences. Here we compared women's preferences for masculine faces at two points in the menstrual cycle where women differed in salivary testosterone, but not in salivary progesterone or estrogen. Preferences for masculinity were strongest when women's testosterone levels were relatively high. Our findings complement those from previous studies that show systematic variation in masculinity preferences during the menstrual cycle and suggest that change in testosterone level may play an important role in cyclic shifts in women's preferences for masculine traits.
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Introduction

Many studies have demonstrated that women's face preferences change during the menstrual cycle. For example, women's preferences for masculine male faces are stronger during the late follicular, fertile phase of the menstrual cycle than during other phases (Frost, 1994; Johnston et al., 2001; Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000). Increased attraction to masculine men during the late follicular phase has also been observed for attractiveness judgments of men's voices (Feinberg et al., 2006; Puts, 2006) and in preferences for video clips of male behavioral displays of dominance (Gangestad et al., 2004). Furthermore, women's preferences for androstenone (a putative male pheromone) are stronger around ovulation than at other times (Grammer, 1993).

Many researchers have suggested that increased attraction to masculine men during the late follicular phase of the menstrual cycle is an adaptation for increasing offspring health (e.g. Gangestad et al., 2004; Johnston et al., 2001; Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000). Indeed, masculine traits in men are thought to signal greater heritable immunity to infectious disease (see Fink and Penton-Voak, 2002; Gangestad and Simpson, 2000 for reviews) and ratings of men's facial masculinity are positively associated with estimates of their long-term health from medical records (Rhodes et al., 2003). Importantly, change in women's preference for masculinity during the menstrual cycle is independent of increased contagion avoidance when raised progesterone levels prepare the body for

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pregnancy (Jones et al., 2005). Changing masculinity preferences are then not explained by pressures to avoid unhealthy males when hormonal patterns are similar to those during pregnancy.

While studies of cyclic shifts in women's preferences for masculine men initially focused on establishing a link between conception risk and preferences (e.g. Frost, 1994; Johnston et al., 2001; Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000), recent studies have examined the hormonal mechanisms that may contribute to this cyclic variation in preferences. These more recent studies have emphasized that, while masculinity preferences are associated with conception risk, conception risk itself cannot be a mechanism for change in women's behavior (Jones et al., 2005; Puts, 2006; see also DeBruine et al., 2005; Fisher, 2004). It is therefore important to identify the proximate (e.g. hormonal) mechanisms for cyclic shifts in women's preferences. Lower progesterone levels during the menstrual cycle have been found to be associated with higher preferences for masculine voices (Puts, 2006) and faces (Jones et al., 2005). By contrast, estrogen level has not been found to be associated with masculinity preferences (Puts, 2006; Jones et al., 2005). While these findings suggest that change in progesterone level may be the hormonal mechanism that underpins cyclic variation in masculinity preferences, Jones et al. (2005) emphasized that changes in other hormone levels might also contribute to cyclic variation in women's face preferences.

Women's testosterone levels change systematically during the menstrual cycle, gradually increasing from menstruation to mid-cycle (i.e. around ovulation) and then slowly declining (Alexander et al., 1990; Bloch et al., 1998; Dabbs and de La Rue, 1991; Morris et al., 1987; Persky et al., 1978; Van Goozen et al., 1997). Furthermore, some studies have reported positive relationships between women's testosterone levels and indices of their sex drive (e.g. Riley and Riley, 2000). Thus, change in testosterone level may contribute to cyclic variation in women's preferences for masculine faces. We therefore tested if the strength of preferences for masculine faces differed between two points in the menstrual cycle differing in salivary testosterone level, but not in salivary progesterone or estrogen levels. If change in testosterone level contributes to cyclic variation in preferences for masculine faces, we predicted that preferences for masculinity would be strongest when salivary testosterone levels are relatively high.

If cyclic shifts in preferences for masculine faces function to increase offspring health (Fink and Penton-Voak, 2002; Gangestad and Simpson, 2000), we might expect cyclic changes to occur only for judgments of male faces (i.e. 'mate-choice relevant' faces, Johnston et al., 2001). However, if cyclic shifts in preferences for masculinity in female faces have a low cost, then they may occur as a functionless by-product of an adaptation for increasing offspring health (Jones et al., 2005). Only two studies have directly compared cyclic shifts in women's preferences for masculinity in male and female faces. Johnston et al. (2001) found cyclic shifts in preferences for masculinity in male faces, but not in female faces. By contrast, Jones et al. (2005) found equivalent variation in preferences for masculine faces during the menstrual cycle for judgments of men's and women's faces. To further investigate the specificity of cyclic variation in women's preferences for masculine faces, here we tested for systematic variation in women's preferences for masculinity in both male and female faces during the menstrual cycle.

Methods

Stimuli

Following previous studies of cyclic variation in women's preferences for masculine faces (Jones et al., 2005; Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000; see also DeBruine et al., 2006; Little et al., 2005), we used prototype-based image transformations to objectively manipulate sexual dimorphism of 2D shape in face images. Although different methods for manipulating masculinity of face images have been used in some other studies (e.g. Johnston et al., 2001), these methods have been shown to produce effects on face perceptions that are equivalent to those produced using the methods used in our current study (DeBruine et al., 2006).

First, male and female prototype (i.e. average) faces were manufactured using established computer graphic methods that have been widely used in studies of face perception (e.g. Jones et al., 2005; Penton-Voak et al., 1999). Prototypes are composite images that are constructed by averaging the shape, color and texture of a group of faces, such as male or female faces. These prototypes can then be used to transform images by calculating the vector differences in position between corresponding points on two prototype images and changing the position of the corresponding points on a third image by a given percentage of these vectors (see Rowland and Perrett, 1995; Tiddeman et al., 2001 for technical details).

Here, 50% of the linear differences in 2D shape between symmetrized versions of the male and female prototypes were added to or subtracted from face images of 20 young White male adults (age: M=19.5 years, SD=2.3) and 20 young White female adults (age: M=18.4 years, SD=0.7). This process creates masculinized and feminized versions of the individual face images that differ in sexual dimorphism of 2D shape and that are matched in other regards (e.g. identity, skin color and texture, Rowland and Perrett, 1995). Examples of masculinized and feminized versions of male and female faces are shown in Fig. 1. Thus, 40 pairs of images were produced in total (each pair consisting of a masculinized and a feminized version of the same individual): 20 pairs of female face images and 20 pairs of male face images.

Manipulation check

We undertook a pilot study to establish whether or not manipulating sexual dimorphism of 2D shape in our stimuli influenced perceptions of their masculinity. Participants (N=38, all female, age: M=20.15 years, SD=3.92) viewed the 40 pairs of face images and were asked to choose the face in each pair that looked more masculine. For each participant, the order of face pairs was fully randomized, as was the side of the screen on which any given image was shown. Note that the two-alternative forced-choice method that we used to assess perceptions of masculinity produces a single score for each participant (the proportion of trials on which the more masculine face in each pair was chosen). One-sample t-tests confirmed that the masculinized versions were chosen as the more masculine face more often than would be predicted by chance for both male ($t(37)=10.52$, $p<0.001$; M=0.87, SE=0.03) and female faces ($t(37)=13.87$, $p<0.001$; M=0.84, SE=0.03), confirming that our image manipulation influenced perceptions of facial masculinity in the predicted way (see also DeBruine et al., 2006).

Procedure

Participants (N=70; age: M=22.38 years, SD=7.48; all reporting no hormonal contraceptive use or pregnancy) were tested on two to four occasions. Each participant was tested once a week at approximately the same time of day (mean unsigned difference between time of day of highest and lowest testosterone test sessions=14.37 min, SD=44.74). The interval between each test session was 1 week. Although the time of day in which test sessions took place differed *across* women, the time of day of test sessions remained constant *within* each woman.

In each test session, participants were shown the 40 pairs of face images (20 male and 20 female) and were asked to choose the face in each pair that was

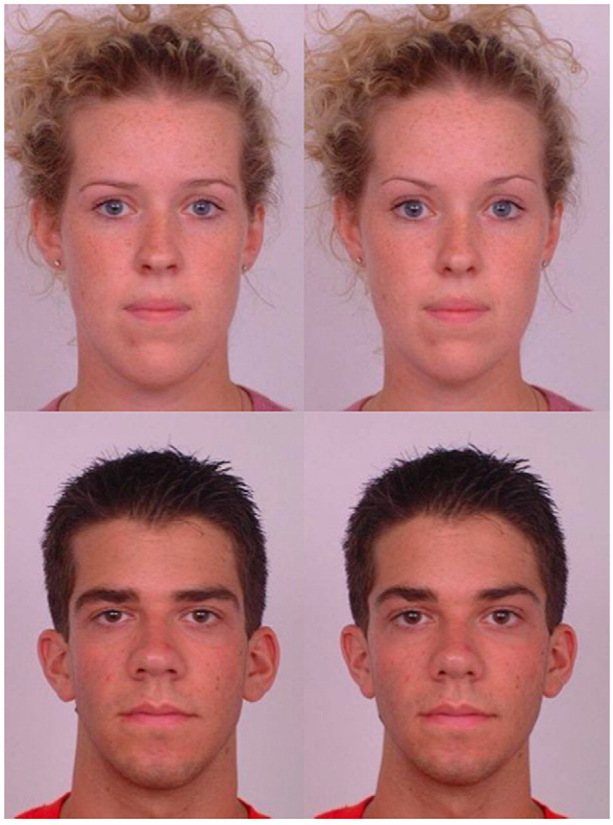

Fig. 1. Examples of masculinized (left column) and feminized (right column) male and female face images. Masculinized and feminized versions differ in sexual dimorphism of 2D face shape only and are matched in other regards (e.g. identity, skin color and skin texture).

more attractive (i.e. we used a two-alternative forced-choice paradigm to assess face preferences). The order in which pairs of faces were shown was fully randomized for each participant and the side of the screen on which any particular image was shown was also randomized.

Participants deposited between 3 and 5 mL of saliva by spitting directly into plastic pharmaceutical vials at the beginning of each testing session. The vials were then sealed and frozen at −20 °C. Participants also reported the date of onset of their last period of menstrual bleeding and the expected date of the onset of their next period of menstrual bleeding.

Hormone assay data

Hormonal assays were performed by the biological sciences lab at Queen Margaret University College (Edinburgh, UK). Salivary testosterone levels were determined by a highly sensitive in-house ELISA method (Al-Dujaili, 2006), that is specifically optimized in relation to concerns about the measurement of the low levels of testosterone present in female saliva (Sharp and Al-Dujaili, 2004). Briefly, saliva samples were first extracted with diethylether and 100 μL aliquots of the re-constituted samples. Standards and controls were then pipetted into the 96-well pre-coated ELISA plate. The procedure was then completed as previously described (Al-Dujaili, 2006). The validity of the assay was confirmed by the good correlation between the results obtained by the in-house ELISA and those assayed by Salimetrics (USA) ELISA kit (R^2=0.95, N=58). Cross-reactivity data with major interfering steroids were minimal (except for DHT=2.3% and androstenedione=4.6%). The average recovery of testosterone in this assay was 104.0% (range 97.5% to 110%). The average intra-assay and inter-assay imprecision was 2.1% and 6.7%, respectively. The formal sensitivity of the assay was 1.24 pg/mL.

Salivary progesterone and estrogen (as estradiol) levels were assayed using Salimetrics ELISA kits. Briefly, standards, controls and unknown samples in duplicates were pipetted into a micro-titer plate that was previously coated with rabbit anti-steroid antibody followed by the addition of horseradish peroxidase conjugate. The assays were then completed as described in the kit manual. Cross-reactivity of both assays with their related compounds was minimal. Intra- and inter-assay coefficient of variation ranged between 2.9–7.6% and 3.5–11.2%, respectively. Recovery of both assays as determined by spiking saliva samples with known quantities of the steroid ranged from 93.0–108.8%. Linearity of the assays after diluting saliva samples up to 16 times ranged from 90.8 to 103.8% recovery. The sensitivity of the progesterone ELISA was 5.0 pg/mL and estradiol ELISA was 1.0 pg/mL.

Initial processing of data

Salivary testosterone data were used to identify the test sessions with the highest and lowest salivary testosterone for each participant. Testosterone levels were significantly higher in the highest testosterone session (M=156.5 pg/mL, SE=11.7) than the lowest testosterone session (M=65.9 pg/mL, SE=5.7; $t(69)=9.84$, $p<0.001$; mean difference=90.6 pg/mL, SE=9.2). These testosterone levels are within population norms for young adult females in this laboratory (see also Deady et al., 2006). However, the highest and lowest testosterone test sessions did not differ in salivary progesterone level ($t(69)=1.01$, $p=0.32$; high testosterone test session: M=101.5 pg/mL, SE=11.4; low testosterone test session: M=86.8 pg/mL, SE=11.0) or in estradiol ($t(69)=0.37$, $p=0.72$; high testosterone test session: M=10.9 pg/mL, SE=0.9; low testosterone test session: M=10.5 pg/mL, SE=1.0). Allocating test sessions to highest and lowest salivary testosterone days did not confound testosterone level and order of testing ($p=0.40$).

As previous studies have found that women's testosterone levels gradually increase from menstruation to mid-cycle (i.e. around ovulation) and then slowly decline during the luteal phase (Alexander et al., 1990; Bloch et al., 1998; Dabbs and de La Rue, 1991; Morris et al., 1987; Persky et al., 1978; Van Goozen et al., 1997), we tested if the predicted day of ovulation was closer to the highest testosterone test session than to the lowest testosterone test session. For the highest testosterone test session, the reported number of days until the onset of the next expected period of menstrual bleeding was used to calculate the estimated number of days either until or since the expected day of ovulation (i.e. 14 days before onset of next menses). These values were then coded as the absolute difference in days from ovulation. Corresponding values were calculated in the same way for the lowest testosterone test sessions. A paired samples t-test showed that the highest testosterone test session was typically closer to the expected day of ovulation (mean unsigned difference between cycle day and ovulation=7.25 days, SE=0.52) than the lowest testosterone test session was (mean unsigned difference between cycle day and ovulation=9.38 days, SE=0.73; $t(64)=-2.131$, $p=0.037$). The diary data we had used to calculate these values were incomplete for 5 participants, who were therefore not included in the analysis of the relationship between salivary testosterone and cycle day.

For each participant, we calculated the proportion of trials on which the more masculine face in each pair of male faces was chosen in the highest testosterone test session. We also calculated the proportion of trials on which the more masculine face in each pair of male faces was chosen in the lowest testosterone test session. Corresponding values for judgments of female faces were also calculated for each test session. All face preference scores were normally distributed (Kolmogorov–Smirnov test: all $p>0.10$).

Results

To test for an effect of testosterone level on masculinity preferences, we compared the proportion of trials on which the masculine faces were preferred in the highest and lowest testosterone test sessions using a repeated measures ANOVA [within-subjects factors: testosterone level (highest, lowest), sex of face (male, female)]. This analysis revealed a main effect of sex of face ($F(1,69)=72.01$, $p<0.001$), whereby masculinity was preferred more often when judging the attractiveness of male faces (mean proportion of trials on which masculinity was preferred=0.40, SE=0.03) than when judging the attractiveness of female faces (mean proportion of trials on which masculinity

was preferred=0.19, SE=0.02). The repeated measures ANOVA also revealed a main effect of testosterone level ($F(1,69)=8.18$, $p=0.006$). Women preferred masculine faces more often in the highest testosterone test session (mean proportion of trials on which masculinity was preferred=0.32, SE=0.02) than in the lowest testosterone test session (mean proportion of trials on which masculinity was preferred=0.27, SE=0.02). The interaction between testosterone level and sex of face was not significant ($F(1,69)=0.42$, $p=0.52$). The main effect of testosterone remained significant when change in progesterone level was included in the analysis as a covariate ($F(1,68)=7.99$, $p=0.006$) and also when change in estradiol was included as a covariate ($F(1,68)=7.62$, $p=0.007$). Paired samples t-tests comparing the proportion of trials on which the masculine faces were chosen in the highest and lowest testosterone test sessions revealed significant differences when judgments of male and female faces were analyzed separately (male faces: $t(69)=2.05$, $p=0.045$; female faces: $t(69)=2.22$, $p=0.030$). These effects of testosterone level are illustrated in Fig. 2.

Further analyses revealed that the women in our study demonstrated a strong general preference for feminine male and female faces (see also Perrett et al., 1998; Penton-Voak et al., 1999). One-sample t-tests, comparing the proportion of trials on which the masculine faces were chosen against chance (i.e. 0.5), showed that feminine versions of faces were chosen more often than their relatively masculine counterparts when male ($t(69)=2.74$, $p=0.008$) and female ($t(69)=14.44$, $p<0.001$) faces were judged in the highest testosterone test session and also when male ($t(69)=4.79$, $p<0.001$) and female ($t(69)=16.85$, $p<0.001$) faces were judged in the lowest testosterone test session. Given this strong general preference for feminine faces, it is possible that increased preference for masculinity when testosterone level is high simply reflects women's preferences regressing to the mean when testosterone level is high.

In light of the above, we carried out a second repeated measures ANOVA to establish whether or not the effect of testosterone level on attraction to masculine faces could be explained solely by women's preferences regressing to the mean (i.e. chance) when testosterone levels were relatively high. By contrast with our main analysis, which analyzed the strength of preferences for masculinity in the high and low testosterone sessions, in this follow-up analysis we analyzed the absolute difference in preference from what would be expected by chance alone (i.e. the extent to which preferences deviated from chance, irrespective of the direction of these preferences). For each condition, the unsigned (i.e. absolute) difference between the proportion of masculine faces chosen and chance (i.e. 0.5) was calculated. These responses were then analyzed using a repeated measures ANOVA [within-subjects factors: testosterone level (highest, lowest), sex of face (male, female)]. This analysis revealed a main effect of sex of face ($F(1,69)=63.40$, $p<0.001$), reflecting the tendency to generally prefer a greater proportion of masculine faces when judging male faces rather than female faces (see earlier analyses). There were no other significant effects (all $F<2.5$, all $p>0.13$), however, demonstrating that the significant effect of testosterone level on preferences for masculine faces in our main analysis was not due to participants' face preferences simply regressing to the mean (i.e. chance) when testosterone level was high.

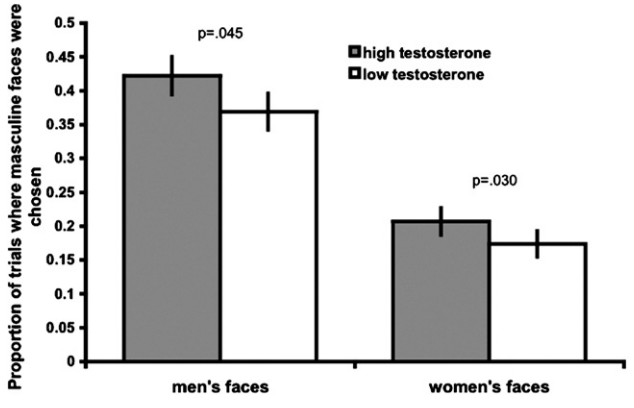

Fig. 2. Increased attraction to masculinity in male and female faces is associated with raised testosterone level. Bars show the mean proportion of trials on which masculine faces were preferred and standard error of the means.

Additional analyses

We compared preferences for masculine faces from the test sessions with the highest and lowest progesterone levels to test if change in progesterone level also contributes to cyclic shifts in masculinity preferences (as has been suggested by previous studies, Jones et al., 2005; Puts, 2006). A repeated measures ANOVA [within-subjects factors: progesterone level (highest, lowest), sex of face (male, female)] revealed a main effect of sex of face (stronger preferences for masculinity in men's than women's faces, $F(1,69)=89.34$, $p<0.001$) and no other significant effects (all $F<0.12$, all $p>0.70$). However, including late follicular estradiol as a covariate revealed a significant three-way interaction among progesterone level, sex of face and late follicular estradiol ($F(1,68)=4.16$, $p=0.045$). Women with high levels of estradiol in the late follicular phase tended to show a greater increase in masculinity preference for men's faces when progesterone levels were relatively low than did women with lower levels of estradiol in the late follicular phase ($r=0.20$, $p=0.098$). No such association between the magnitude of change in masculinity preference and late follicular estradiol was apparent for women's faces ($r=-0.12$, $p=0.320$). Highest and lowest progesterone test sessions differed in both progesterone level ($t(69)=9.88$, $p<0.001$; high progesterone session: M=169.5 pg/mL; SE=14.5; low progesterone session: M=43.2 pg/mL; SE=4.0) and estradiol ($t(69)=5.95$, $p<0.001$; high progesterone session: M=13.1 pg/mL; SE=1.1; low progesterone session: M=7.7 pg/mL; SE=0.7), but not in testosterone level ($t(69)=-0.40$, $p=0.681$; high progesterone session: M=105.1 pg/mL; SE=9.7; low progesterone session: M=110.1 pg/mL; SE=11.5).

A further repeated measures ANOVA comparing preferences from the test sessions with the highest and lowest levels of estradiol [within-subjects factors: estradiol level (highest, lowest), sex of face (male, female)] also revealed a main effect

of face sex ($F(1,69)=86.98$, $p<0.001$) and no other significant effects (all $F<0.15$, all $p>0.29$). Including late follicular estradiol as a covariate did not reveal a three-way interaction among estradiol level (high, low), sex of face and late follicular estradiol ($F(1,68)=1.28$, $p=0.26$). Highest and lowest estradiol test sessions differed in both estradiol ($t(69)=11.05$, $p<0.001$; high estradiol session: M=16.2 pg/mL; SE=1.1; low estradiol session: M=6.5 pg/mL; SE=0.6) and progesterone level ($t(69)=5.24$, $p<0.001$; high estradiol session: M=122.6 pg/mL; SE=14.3; low estradiol session: M=56.4 pg/mL; SE=6.1), but not in testosterone level ($t(69)=0.99$, $p=0.328$; high estradiol session: M=115.4 pg/mL; SE=9.5; low estradiol session: M=105.8 pg/mL; SE=10.7).

Discussion

Our findings show that women have stronger preferences for masculine faces when their salivary testosterone levels are high than when their salivary testosterone levels are relatively low. Furthermore, test sessions that were characterized by high salivary testosterone levels were estimated to be closer to ovulation than test sessions that were characterized by relatively low salivary testosterone levels (see also Alexander et al., 1990; Bloch et al., 1998; Dabbs and de La Rue, 1991; Morris et al., 1987; Persky et al., 1978; Van Goozen et al., 1997). Thus, the observed association between raised testosterone level and increased attraction to masculine faces complements findings from other studies that found increased preferences for masculinity in male faces (Frost, 1994; Johnston et al., 2001; Jones et al., 2005; Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000), voices (Feinberg et al., 2006; Puts, 2006), odors (Grammer, 1993), and behavioral displays of dominance (Gangestad et al., 2004) around ovulation.

Most previous studies of changes in women's preferences for masculine faces during the menstrual cycle either tested only for changes in women's preferences for masculine *men* (e.g. Penton-Voak et al., 1999; Penton-Voak and Perrett, 2000) or did not find cyclic shifts in preferences for masculine female faces (Johnston et al., 2001; but see Jones et al., 2005). However, here we found that women's preferences for masculinity in both male and female faces were strongest when testosterone levels were relatively high. This latter finding suggests that cyclic variation in women's face preferences influences social attitudes to both men and women (see also Jones et al., 2005).

The significant association between raised testosterone level and increased attraction to masculine faces in our study cannot be explained by a general response bias, whereby women's face preferences simply regressed to the mean (i.e. chance) when their testosterone levels were relatively high. This is consistent with findings that preferences for masculine faces are stronger around ovulation than at other times, regardless of whether the sample exhibits a general preference for more masculine than average faces (e.g. Johnston et al., 2001) or more feminine than average faces (e.g. Penton-Voak et al., 1999).

Previous studies that have found associations between low progesterone level and increased attraction to masculinity during the menstrual cycle have interpreted these findings as evidence that change in progesterone level contributes to cyclic variation in women's preferences for masculine traits (Jones et al., 2005; Puts, 2006). Here, however, we observed a significant change in attraction to masculine faces between two points in the menstrual cycle that differed in salivary testosterone level, but not in salivary progesterone level or estradiol, and that was robust to controlling for the effects of change in progesterone and estradiol. This suggests that change in testosterone level may play an important role in within-subject variation in women's preferences for masculine faces. Although the design of our study allowed us to demonstrate an association between raised testosterone level and the strength of women's masculinity preferences independently of progesterone and estradiol, possible effects of other hormones that may be associated with testosterone remain to be investigated.

Consistent with findings from previous studies of the hormonal mechanisms that may contribute to cyclic variation in masculinity preferences (Jones et al., 2005; Puts, 2006), we found no evidence that change in estradiol is associated with changes in women's preferences for masculine traits. By contrast with findings from these previous studies, however, we also observed no main effect of progesterone level on masculinity preferences. While it is possible that previous findings for change in progesterone level arose because some phases of the menstrual cycle that are characterised by low progesterone level are also characterised by relatively high levels of testosterone, inconsistent findings for progesterone level among studies could also occur if factors other than change in hormone levels qualify the magnitude of cyclic shifts in masculinity preferences (e.g. Johnston et al., 2001). Indeed, further analyses revealed a significant three-way interaction among progesterone level, sex of face and late follicular estradiol: Women with high levels of estradiol in the late follicular phase tended to show a greater increase in masculinity preference for men's (but not women's) faces when progesterone levels were relatively low than did women with lower levels of estradiol in the late follicular phase. This interaction complements Johnston et al. (2001), who found that women who scored low on a test assessing masculinity of attitudes showed greater cyclic shifts in preferences for masculinity in men's faces than did women with relatively high scores, and suggests that the role of change in progesterone level for cyclic shifts in masculinity preferences may be more complex than was previously thought.

Increased testosterone level in women has been implicated in reduced mimicry of facial expressions (Hermans et al., 2006) and increased sensitivity to angry facial expressions (van Honk et al., 1999). Here we extend these findings for effects of testosterone level on responses to facial expressions by demonstrating that changes in women's testosterone levels are also associated with changes in their face preferences. Furthermore, while previous studies of systematic variation in women's preferences for masculine individuals during the menstrual cycle have emphasized the importance of change in progesterone level (Jones et al., 2005; Puts, 2006), our findings suggest that change in testosterone level may contribute to cyclic shifts in women's masculinity preferences.

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