Changes in salivary estradiol predict changes in women's preferences for vocal masculinity

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Abstract

Although many studies have reported that women's preferences for masculine physical characteristics in men change systematically during the menstrual cycle, the hormonal mechanisms underpinning these changes are currently poorly understood. Previous studies investigating the relationships between measured hormone levels and women's masculinity preferences tested only judgments of men's facial attractiveness. Results of these studies suggested that preferences for masculine characteristics in men's faces were related to either women's estradiol or testosterone levels. To investigate the hormonal correlates of within-woman variation in masculinity preferences further, here we measured 62 women's salivary estradiol, progesterone, and testosterone levels and their preferences for masculine characteristics in men's voices in five weekly test sessions. Multilevel modeling of these data showed that changes in salivary estradiol were the best predictor of changes in women's preferences for vocal masculinity. These results complement other recent research implicating estradiol in women's mate preferences, attention to courtship signals, sexual motivation, and sexual strategies, and are the first to link women's voice preferences directly to measured hormone levels.

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Introduction

Recent meta-analyses suggest that women's preferences for masculine men are stronger during the late follicular (i.e., high-fertility) phase of the menstrual cycle than during the early follicular or luteal (i.e., low-fertility) phases (Gildersleeve et al., in press; but see Wood et al., 2014). For example, this pattern of results has been reported in studies of women's preferences for men's faces (Johnston et al., 2001; Penton-Voak et al., 1999), bodies (Little et al., 2007), voices (Feinberg et al., 2006; Puts, 2005), body odors (Havlíček et al., 2005), and behavioral displays (Gangestad et al., 2004). Researchers have suggested that increased preferences for masculine men during the fertile phase of the menstrual cycle may function to increase offspring health (Gangestad and Thornhill, 2008) and/or dominance (Scott et al., 2013).

The majority of studies investigating changes in women's masculinity preferences during the menstrual cycle have simply compared preferences between high-fertility and low-fertility phases (Gildersleeve et al., in press). Far fewer studies have addressed the hormonal mechanisms that may underpin these cyclic shifts in women's mate preferences. Initial research on this topic examined women's estimated hormone levels by converting information about each participant's position in the menstrual cycle at test to estimated hormone levels using actuarial tables. These studies reported negative correlations between estimated progesterone levels and women's facial (Jones et al., 2005) and vocal (Puts, 2006) masculinity preferences. More recent work has extended this early research by measuring estradiol, testosterone, and progesterone levels from saliva (Bobst et al., 2014; Roney and Simmons, 2008; Roney et al., 2011; Welling et al., 2007). These studies found that women's preferences for sexually dimorphic and/or androgen-dependent characteristics in men's faces were positively correlated with either their salivary estradiol (Roney and Simmons, 2008; Roney et al., 2011) or testosterone (Bobst et al., 2014; Welling et al., 2007) levels, both of which can show mid-cycle peaks (Dabbs and de La Rue, 1991; Sherman and Korenman, 1975). These inconsistent results indicate that further research is required to elucidate the hormonal mechanisms that might contribute to within-woman variation in masculinity preferences.

Previous studies investigating the possible relationships between measured salivary hormone levels and women's masculinity preferences have focused exclusively on women's judgments of men's facial attractiveness. However, masculine characteristics are also known to be important factors for women's perceptions of men's voices, with women perceiving men with masculine voices as both attractive and physically dominant (reviewed in Feinberg, 2008; Puts, 2010).
Although previous studies have shown that women's preferences for masculinized versus feminized versions of men's voices are stronger during the fertile phase of their menstrual cycle (Feinberg et al., 2006; Puts, 2005), no previous studies have used direct measures of women's hormone levels to investigate the hormonal correlates of within-woman changes in preferences for men's vocal masculinity. Additionally, previous studies investigating the hormonal correlates of preferences for experimentally-manipulated vocal masculinity (Feinberg et al., 2006; Puts, 2005) assessed women's preferences for vocal masculinity by simultaneously altering two anatomically and acoustically distinct sexually dimorphic characteristics in recordings of men's voices: voice pitch (i.e., the perception of fundamental frequency and/or corresponding harmonics, Titze, 1994) and formants (i.e., the resonant frequencies of the supralaryngeal vocal-tract and an index of body size, Fant, 1960; Titze, 1994). This is potentially noteworthy, because pitch and formants are known to have independent effects on women’s judgments of men’s vocal attractiveness (Feinberg et al., 2005; Pisanski and Rendall, 2011) and both masculine pitch and masculine formants are correlated with circulating testosterone levels in men (Bruckert et al., 2006; Dabbs and Mallinger, 1999). Other studies investigating the hormonal correlates of women’s preferences for vocal masculinity did not use experimental methods to assess preferences, but calculated the correlation coefficient between naturally occurring variation in voice pitch and each woman’s attractiveness ratings of these voices (Puts, 2006). Consequently, the relative contribution of voice pitch and formant frequencies to hormone-linked variation in vocal masculinity preferences is unclear.

In light of the above, we investigated the hormonal correlates of within-woman variation in preferences for masculine versus feminine pitch and masculine versus feminine formants in recordings of men’s voices. Women (none of whom were using any form of hormonal supplement, such as estradiol) did not investigate the hormonal correlates of cyclic shifts between fertile and non-fertile women’s masculinity preferences, but did find that women with higher average (i.e., trait) estradiol tended to show smaller cyclic shifts between fertile and non-fertile phases in their masculinity preferences.

had not taken any form of hormonal supplement in the previous 90 days.

Voice stimuli

Recordings of 6 men between the ages of 18 and 25 speaking the English monophthong vowels, “ah” / /, “ee” / /, “e” / /, “oh” / /, and “oo” / /, were made in an anechoic sound-controlled booth. Recordings were made using a Sennheiser MKH 800 condenser microphone with a cardioid pick-up pattern and at an approximate distance of 5–10 cm. Audio was digitally encoded with an M-Audio Fast Track Ultra interface at a sampling rate of 96 kHz and 32-bit amplitude quantization, and stored onto a computer as PCM WAV files using Adobe Soundbooth CS5 version 3.0. The number of voices used in our study is similar to the numbers used in previous studies examining voice preferences (e.g., Feinberg et al., 2008a; Pisanski and Rendall, 2011; Riding et al., 2006), the results of which generalize well to studies using larger samples of voices (e.g., Feinberg et al., 2008b; Puts, 2005).

We created two masculinized and two feminized versions of each original voice recording by independently manipulating pitch or formants using the Pitch-Synchronous Overlap Add (PSOLA) algorithm in Praat version 5.2.15 (Boersma and Weenink, 2013; Mouloune and Charpentier, 1990). The PSOLA method allows one voice feature (e.g., pitch or formants) to be manipulated while leaving other voice features unaltered, and has been used successfully in many past studies of voice perception in humans (Feinberg et al., 2005, 2008a; Jones et al., 2010) and other mammals (Chazanfar et al., 2007; Reby et al., 2005). Following results of psychophysical experiments identifying the optimal level of manipulation for studies of the attractiveness of acoustic properties of human speech (e.g., Re et al., 2012), we raised or lowered pitch by 10% from baseline while holding formants constant (pitch masculinity manipulation) and raised or lowered formants by 10% from baseline while holding pitch constant (formant masculinity manipulation). This process created 6 pairs of male voices that differed in pitch and 6 pairs of male voices that differed in formants. Work by Pisanski and Rendall (2011) suggests that percent-based manipulations of pitch and formants are perceptually equivalent.

The mean fundamental frequencies and formant frequencies of masculinized and feminized voices, given in Table 1, span the natural ranges of frequencies for large samples of English vowel sounds spoken by adult males (Bruckert et al., 2006; Feinberg et al., 2008b; Puts et al., 2012; Rendall et al., 2005). Following masculinity manipulation, we amplitude normalized the sound pressure level of all voices to 70 dB using the root mean squared method.

Masculinity manipulation check

We conducted a manipulation check to verify that masculinized voice stimuli influenced women’s perceptions of men’s masculinity

### Table 1

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>F0 (Hz)</th>
<th>F1 (Hz)</th>
<th>F2 (Hz)</th>
<th>F3 (Hz)</th>
<th>F4 (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masculinized pitch</td>
<td>111</td>
<td>457</td>
<td>1525</td>
<td>2567</td>
<td>3440</td>
</tr>
<tr>
<td>Feminized pitch</td>
<td>135</td>
<td>460</td>
<td>1525</td>
<td>2571</td>
<td>3437</td>
</tr>
<tr>
<td>Masculinized formants</td>
<td>123</td>
<td>421</td>
<td>1375</td>
<td>2351</td>
<td>3145</td>
</tr>
<tr>
<td>Feminized formants</td>
<td>123</td>
<td>513</td>
<td>1682</td>
<td>2817</td>
<td>3756</td>
</tr>
</tbody>
</table>

Acronyms: F0 = fundamental frequency (pitch); F1-F4 = first to fourth formant; Fm = mean formant frequency (an average of F1-F4). Mean F0 was measured using Praat's autocorrelation algorithm with a search range set to 65–300 Hz. Formants F1–F4 were measured using the Burg Linear Predictive Coding algorithm. Formants were first overlaid on a spectrogram and manually adjusted until the best visual fit of predicted onto observed formants was obtained. All acoustic measurements were taken from the central, steady-state portion of each vowel, averaged across vowels for each voice, and then averaged across voices. This was done separately for each type of masculinity manipulation.
and dominance. Twenty-seven women (mean age = 24.56 years, SD = 6.55 years) listened to the 12 pairs of voices (each pair consisting of a masculinized and a feminized version of the same voice) and indicated which voice in each pair sounded more masculine. A different group of 27 women (mean age = 22.77 years, SD = 5.74 years) listened to the same voices and indicated which voice in each pair sounded more dominant. Trial order and the order in which participants listened to the masculinized and feminized versions in each pair were fully randomized. None of the women who took part in the manipulation check participated in the main study.

One-sample t-tests showed that, overall, the proportion of trials on which women chose the masculinized voices as the more masculine or dominant was significantly greater than what would be expected by chance alone (masculinity: \( t_{25} = 16.91, p < .001, M = .90, SEM = .02 \); dominance: \( t_{25} = 3.02, p < .001, M = .86, SEM = .03 \)). Additional one-sample t-tests showed the same pattern of results when we separately analyzed voices manipulated in either formants only (masculinity: \( t_{25} = 13.30, p < .001, M = .90, SEM = .03 \); dominance: \( t_{25} = 10.22, p < .001, M = .86, SEM = .04 \)) or pitch only (masculinity: \( t_{25} = 15.56, p < .001, M = .90, SEM = .03 \); dominance: \( t_{25} = 9.45, p < .001, M = .85, SEM = .03 \)). Together, these results indicate that our voice stimuli differed reliably in both perceived masculinity and dominance, and that stimuli with lowered pitch and stimuli with lowered formants elicited analogous perceptions of masculinity and dominance. These results replicate those in prior studies (e.g., Feinberg et al., 2005; Pisanski and Rendall, 2011).

### Coding of masculinity preference data

Following previous studies (e.g., Feinberg et al., 2008a), preference scores were coded as follows:

- **0** = feminine voice rated 'much more attractive' than masculine voice
- **1** = feminine voice rated 'more attractive' than masculine voice
- **2** = feminine voice rated 'somewhat more attractive' than masculine voice
- **3** = feminine voice rated 'slightly more attractive' than masculine voice
- **4** = masculine voice rated 'slightly more attractive' than feminine voice
- **5** = masculine voice rated 'somewhat more attractive' than feminine voice
- **6** = masculine voice rated 'more attractive' than feminine voice
- **7** = masculine voice rated 'much more attractive' than feminine voice.

These preference scores were then used to calculate two different masculinity preference measures for each participant. The first was a **formant masculinity preference measure** in which scores were averaged from the 6 trials on which voices manipulated only in formants were presented. The second was a **pitch masculinity preference measure** in which scores were averaged from the 6 trials on which voices manipulated only in pitch were presented. Higher scores on these masculinity preference measures indicate stronger masculinity preferences. Preference measures were calculated separately for each of the five test sessions and were the dependent variable in our analyses.

### Results

We first tested whether the women in our sample, on average, preferred masculinized versions of male voices over feminized versions. To do this we used one-sample t-tests to compare each woman's average masculinity preference (i.e., her masculinity preference averaged across all test sessions) with what would be expected by chance alone (3.5). Analyses of the formant masculinity preference measure (\( \overline{M}_1 = 6.23, p = .001, M = 4.03, SEM = .09 \)) and the pitch masculinity preference measure (\( \overline{M}_2 = 10.02, p = .001, M = 4.17, SEM = .07 \)) both demonstrated that masculinity preferences were significantly above chance. Average formant and pitch masculinity preferences were positively correlated (r = .42, n = 62, p < .001). Older women tended to have higher scores on the formant masculinity preference (r = .23, n = 62, p = .070) and pitch masculinity preference (r = .22, n = 62, p = .082) measures. However, neither of these relationships was significant.

We used multilevel modeling to test for within-subject effects of hormone levels on vocal masculinity preferences. Analyses were conducted using R (R Core Team, 2013), lme4 (Bates et al., 2014), and lmerTest (Kuznetsova et al., 2013). Masculinity preference scores served as our dependent variable. The intercept was allowed to vary by participant and also by participant’s test session. For each test session, each participant provided two vocal masculinity preference scores: one for formants and one for pitch. Consequently, manipulation type (0 = formant, 1 = pitch) was entered for each score and testosterone, estradiol, progesterone, and estradiol-to-progesterone ratio (each centered on their grand means) were entered for each test session to test for independent within-subject effects of these hormones. All four interactions between manipulation type and each hormone level were also entered for each test session. Following an instruction from the editor, session number (1–5) was entered for each test session to control for possible order effects. We also entered participant age (centered on its grand mean) for each participant to control for possible effects of age on masculinity preferences (Little et al., 2010). All four interactions between age and each hormone level were entered for each test session to control for age-related changes in the magnitude of hormonal changes during the

### Hormonal assays

Saliva samples were frozen immediately and stored at −32 °C until being shipped, on dry ice, to the Salimetrics Lab (Suffolk, UK) for analysis. Participants were instructed to avoid consuming alcohol and coffee in the 12 h prior to participation and to avoid eating, drinking, chewing gum, or brushing their teeth in the 60 min prior to participation. Samples were assayed by Salimetrics using the Salivary Estradiol Enzyme Immunoassay Kit 1-3702 (mean = 4.73 pg/mL, SD = 0.91 pg/mL, intra-assay coefficient of variation (CV) = 7.13%, inter-assay CV = 7.45%), Salivary Progesterone Enzyme Immunoassay Kit 1-1502 (mean = 157.25 pg/mL, SD = 70.80 pg/mL, intra-assay CV = 6.20%, inter-assay CV = 7.55%), and Salivary Testosterone Enzyme Immunoassay Kit 1-2402 (mean = 84.69 pg/mL, SD = 18.04 pg/mL, intra-assay CV = 4.60%, inter-assay CV = 9.83%). All assays passed Salimetrics’ quality control. Because estradiol-to-progesterone ratio is correlated with fertility (Baird et al., 1991; Landgren et al., 1980) and some researchers have suggested that women’s masculinity preferences may covary with estrogen-to-progesterone ratio (e.g., Frost, 1994), we also calculated estradiol-to-progesterone ratio (mean = .052, SD = .086) from women’s estradiol (in pg/mL) and progesterone (in pg/mL) data.
menstrual cycle (Lee et al., 1988; Sherman and Korenman, 1975). This initial analysis revealed no interactions between participant age and any hormone levels (all $t < 1.28$, all $p > .20$) and no interactions between manipulation type and any hormone levels (all $t < 1.41$, all $p > .16$) except progesterone ($t = 2.60$, $p = .010$). The full results for this model (and the equations) are given in the Supplemental materials.

Next, all non-significant interactions were removed from the model. This reduced model revealed a near-significant positive effect of estradiol ($t = 1.92, p = .055$), a significant positive effect of participant age ($t = 2.16, p = .034$), a significant negative effect of session number ($t = -2.28, p = .023$), and a significant positive effect of manipulation type ($t = 2.99, p = .003$), whereby masculinity preference scores were greater for the pitch manipulation than they were for the formant manipulation. This model also showed a significant interaction between manipulation type and progesterone ($t = 2.54, p = .011$). The effect of progesterone on preference for masculine formants was negative ($t = -1.53, p = .13$) and the effect of progesterone on preference for masculine pitch was positive ($t = 1.48, p = .14$). However, neither of these effects was significant. There were no significant effects of testosterone ($t = -0.32, p = .75$), or estradiol-to-progesterone ratio ($t = 0.41, p = .68$).

An anonymous reviewer asked that we demonstrate that the observed effect of estradiol was not specific to analyses that controlled for the effects of other hormone levels. Consequently, we conducted an additional analysis including only estradiol, session number, manipulation type, and participant age. As in the analysis described above, there was a near-significant positive effect of estradiol ($t = 1.96, p = .050$), a significant positive effect of participant age ($t = 2.21, p = .031$), a significant negative effect of session number ($t = -2.25, p = .025$), and a significant positive effect of manipulation type ($t = 2.97, p = .003$).

The equations for all of the models reported above are given in our Supplemental materials. Repeating all of these analyses without participant age did not alter the pattern of results.

Discussion

Consistent with previous studies (e.g., Feinberg et al., 2005; Pisansk and Rendall, 2011), women generally preferred recordings of men’s voices with masculinized pitch to versions with feminized pitch and generally preferred recordings of men’s voices with masculinized formants to versions with feminized formants. Furthermore, analyses showed that women’s preferences for masculine pitch and formants in men’s voices tended to be stronger in test sessions where salivary estradiol level was high ($p = .050$ when no other hormones were included in the model and $p = .055$ when all other hormones were included in the model). These results complement findings from recent studies linking estradiol to women’s preferences for androgen-dependent characteristics in men’s faces (Roney and Simmons, 2008; Roney et al., 2011), attention to courtship signals (Rosen and López, 2009), sexual motivation (Roney and Simmons, 2013), and mating strategy (Durante and Li, 2009).

While our results are consistent with previous studies linking estradiol to women’s preferences for masculine characteristics in men’s faces (Roney and Simmons, 2008; Roney et al., 2011), no evidence for a significant relationship between testosterone level and women’s masculinity preferences was observed in the current study. Further research is needed to establish why some studies of variation in women’s masculinity preferences have found that masculinity preferences are predicted by estradiol levels (Roney et al., 2011; Roney and Simmons, 2008; the current study), while others have found that masculinity preferences are predicted by testosterone levels (Bobst et al., 2014; Welling et al., 2007).

Many researchers have suggested that systematic variation in women’s preferences for masculine men during the menstrual cycle functions primarily to increase the likelihood that women mate with masculine men at points during each cycle when conception risk is particularly high (Johnston et al., 2001; Penton-Voak et al., 1999). Other researchers have suggested that within-cycle changes in masculinity preferences are byproducts of mechanisms that function primarily to increase women’s preferences for masculine men during ovulatory cycles, compared to anovulatory cycles (Roney and Simmons, 2008). Ovulatory cycles are characterized by higher estradiol levels (Hambridge et al., 2013), while estradiol-to-progesterone ratio is a good predictor of within-cycle variation in conception risk (Baird et al., 1991; Landgren et al., 1980). Thus, our data linking masculinity preferences to estradiol, rather than estradiol-to-progesterone ratio, may support Roney and Simmons’ (2008) proposal.

We also found little evidence for links between masculinity preferences and progesterone, therefore failing to support the suggestion that changes in women’s masculinity preferences partly reflect increased attraction to prosocial men when raised progesterone level prepares the body for pregnancy (Jones et al., 2005). Studies that estimated hormone levels by converting information about each woman’s position in the menstrual cycle at test to estimated hormone levels using actuarial tables have reported negative correlations between estimated progesterone level and women’s masculinity preferences (Jones et al., 2005; Puts, 2006). However, the current study’s null results for progesterone add to a growing body of evidence suggesting that this pattern does not occur when participants’ hormone levels are measured from saliva (Bobst et al., 2014; Roney and Simmons, 2008; Welling et al., 2007).

In conclusion, here we present the first evidence that within-woman changes in measured salivary hormone levels during the menstrual cycle predict changes in their preferences for masculine men’s voices. Our analyses suggest that estradiol may be the primary hormonal correlate of within-woman variation in preferences for masculine voices, compared with progesterone, testosterone, and estrogen-to-progesterone-ratio. While further research is needed to establish whether estradiol has a direct and/or causal effect on women’s mate preferences, our findings add to a growing body of evidence linking estradiol to women’s mate preferences, sexual motivations, and sexual strategy (Durante and Li, 2009; Roney and Simmons, 2008; Roney et al., 2011). Our findings also complement a growing body of evidence suggesting that estradiol may play an important role in female sexual motivation and mate preferences in non-human mammals (see, e.g., Roney et al., 2011 and Wallen, 2013).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2014.07.006.

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