Hormonal responses to male natural odor among single women with different relationship status

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1. Introduction

Mating preferences and initiation of courtship involves a complex network of factors (Aron et al. 2000, Gangestad et al. 2006). Miller and Maner (2009) suggest that human mating is guided by relatively subtle processes, which take place at the molecular level. Some subtle factors shaping the mating behavior of other animal species may also affect human beings (Buss 1989). Chemosensory communication via pheromones is used by animals to transmit behaviorally relevant information, such as sexual status, social organization and danger (Pause 2004). Chemosensory communication also occurs in humans, and may play an important role influencing mating-related decisions (Singh and Bronstad 2001, Miller and Maner 2009).

Although the role of pheromones is controversial, it is understood that odors and chemicals play an important part in human relationships, for both sexes. Human olfaction is sensitive to certain kinds of chemosensory stimuli from the opposite sex. These cues can have an effect on a woman's physiological and endocrine responses. The different relationship status of the subjects also contributes to differences in women’s testosterone levels (van Anders and Watson 2007, Hamilton and Meston 2010). The purpose of this research was to compare the testosterone response in single women in and out of a relationship when exposed to male scents.
Materials and Methods:

Male Natural Odor Collection

Four college male student age 22 ± 2 years were recruited for scent collection. The procedure for scent collection was similar to previous studies (Singh and Bronstad 2001, Miller and Maner 2009). Men wore a T-shirt to bed for two nights. During the day the T-shirt was placed in a sealed freezer bag and refrigerated at 4 °C until used the next night. During the study, the men showered each day with unscented soap and shampoo, avoided use of cologne, deodorants and antiperspirants, refrained from eating odor-producing food, smoking, drinking alcohol, using drugs, and abstained from sexual activity or sleeping with someone else in the same bed.

Subjects

The study was approved by the Brigham Young University-Hawaii Institutional Review Board (IRB). Twenty Brigham Young University- Hawaii female students were recruited. All subjects were single women, age 21 ± 3 years. Exclusion criteria included chronic use of medication (including oral contraception, alcohol and tobacco), a history of head or nasal passage trauma, and a history of repeated or current sinus infections. Since menstrual cycle could
affect women’s olfactory acuity, all subjects were tested on the 14th day of their menstrual cycle (the first day of menstruation was counted as day 1).

Sample Collection

To reduce environmental hormonal interference, a female experimenter interacted with each volunteer. The subject signed a consent and the experimenter explained to her the procedures and instructions for exposure to the T shirt samples.

After subjects spent twenty minutes in a constant environment (temperature 23°C), a baseline saliva sample was taken with a salimetrics oral swab. The swap was labeled, returned to the plastic container, and stored at -20°C.

After the collection of the samples, the same-sex experimenter held the experimental bag under each subject’s nose. The subject was instructed to take six deep sniffs. The experimenter had no verbal interaction with the subjects. The subject rated compound intensity, pleasantness and familiarity on a 1-9 point scale. After twenty minutes, another saliva sample was collected.

Data Process and Analysis

The saliva samples were sent to Salimentrics Laboratory for an analysis of testosterone by ELISA. The data were analyzed by Paired T-Test and One Way ANOVA using the
General Linear Model (GLM).

Results:

There were significant testosterone responses to the natural Male-Scent ($p = 0.046$), but the testosterone values were not different for the status of relationship ($p=0.619$), perceived intensity ($p=0.440$), pleasantness ($p=0.402$), or familiarity ($p=0.153$).

The Paired-T Test comparing the Control subjects’ testosterone concentration before and after testing was not significantly different (figure 1). The mean for Control before testing was 85.77 (SD=28.43), and 89.85 (SD=29.56) for the Control after testing.

Figure 1. The mean and standard deviation for testosterone concentration of the Control group before and after the exposure to T-shirt. There was no statistical difference between the two groups ($p=0.460$).

A Two-Sample T Test was performed to compare the testosterone concentration between the control and the test subjects before testing. The p-value ($p=0.393$) indicated that there was
no significant difference between the two groups. The mean for the control was 85.8 (SD=28.4), and the mean was 95.6 (SD=21.3) for the test subjects (figure 2).

Figure 2. The mean and standard deviation for testosterone concentration of the control group and the test group before the start of the experiment. There was no statistical difference between groups (p=0.393).

There was a significant difference (p=0.035) for Male-Scented subjects’ testosterone concentration after exposure to the male scent. The mean before Male-Scented exposure was 95.64 (SD=21.31), while after the mean was 85.28 (SD=15.28). The testosterone concentrations were significantly lower following exposure to the male scent (figure 3).
Eight of the twenty subjects were in relationships (R), and the other 12 were not (NR).

The mean for the NR group after the experiment was 84.8 (SD= 22.4) while those in relationship averaged 91.7 (SD= 24.9). The P-value from Two-Sample T-Test was 0.536, which indicated the difference, was not significant (figure 4). The relationship status had no effect on the change in testosterone level.
Figure 4. The mean and standard deviation for testosterone concentration of NR and R groups after the exposure to T-shirt. There was no significant statistical difference between groups \( (P=0.536) \).

The General Linear Model was used to determine the significance of perceived intensity, pleasantness, and familiarity during compound presentation on the testosterone level. Each P-value was higher than 0.05 \( (P_{\text{Intensity}}=0.440, P_{\text{Familiarity}}=0.402, P_{\text{Pleasantness}}=0.153) \), indicating that there were no significant differences by perceived intensity, pleasantness, and familiarity.

**Discussion:**

This present study examined the hormonal responses to male natural scent among single women in and out of relationships. The result confirmed the hypothesis that male natural scent could alter the testosterone level among single women. The difference in relationship status was not significant.
Perceived impressions of the scent showed slight inconsistencies with the study of Bensafi et al. (2004). In this study, rating on perceived intensity show no correlation to the alteration of testosterone levels among single women, contrary to the results of Bensafi et al. (2004). These results do concur with Bensafi et al. (2004) that the familiarity and pleasantness have no effect. Differences were noticed in the procedures of Bensafi et al. (2004). In their study, an opposite sex experimenters presented the samples in a jar to the subjects, in this study a same-sex experimenter presented the sample in double zipped Ziploc bag.

A careful study of the experimental methods could explain the differences found in the studies. According to van Anders and Watson (2007) and Hamilton and Meston (2010), the different relationship status of the subjects contributed to differences in the women’s testosterone levels. Both study teams collected saliva samples from subjects in different relationship status after two week of being away from their partners, a day before and the day after they reunited with their partners and had sexual intercourse. Since androgens are closely related to sex drive in women, it is reasonable to assume that changes would occur in testosterone levels. However, the criteria and procedure of this research was different. Only the olfactory sensitivity of male scent was measured. Also, both subjects in long distance relationships and in same city relationships were together, rather than classifying them separately. Therefore, due to the different benchmarks set by the experimenters, and the small sample size of the present research, more
investigation is needed to confirm whether the relationship status contributes to the change of testosterone among single females.

The declined of testosterone was inconsistent with the findings of Bensafi et al. (2004) and Wyart, C. et al. (2007). In these studies, the human chemosignal molecule 4,16-androstadien-3-one (AND) found in male sweat increased women testosterone levels. The time of sample collection may have contributed to the differences found in this study. Bensafi et al. (2004) sampled throughout the ovarian cycle. The current study only sampled in the late afternoon during ovulation. Wyart et al. (2007), collected samples during ovulation and found an increase in testosterone. Their samples were collected in the morning. Studies indicate that testosterone concentration may vary during the day (WebMD 2010).

To conclude, female olfaction is capable to detect the chemosensory stimuli in male natural scent, which alters the female subjects’ hormonal responses. But the relationship status shows no differences in subject’s testosterone levels.

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