Evidence that androstdienone, a putative human chemosignal, modulates women’s attributions of men’s attractiveness

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Considerable research effort has focused on whether specific compounds found within human body odor influence the behavior or physiology of other individuals. The most intensively studied is 4,16-androstadien-3-one, a chemical which is known to modulate mood and have activational effects in the sympathetic nervous system in a context-dependent manner, but whose action in mate choice contexts remains largely untested. Here we present evidence that this androgen steroid may modulate women’s judgments of men’s attractiveness in an ecologically valid context. We tested the effects of androstadienone at a speed-dating event in which men and women interacted in a series of brief dyadic encounters. Men were rated more attractive when assessed by women who had been exposed to androstadienone, an effect that was seen in two out of three studies. The results suggest that androstadienone can influence women’s attraction to men, and also that research into the modulatory effects of androstadienone should be made within ecologically valid contexts.

Androstadienone, chemosignal, attractiveness, speed-dating, olfaction, human mate choice, pheromone
Introduction

Odorous chemicals originating from an animal constitute a rich and potent source of potential information to conspecifics. This information can include sex, identity, relatedness and individual condition, and can be gleaned from glandular sources and urine or fecal scent marks (Gosling and Roberts, 2001). Despite much work on non-human animals, the extent to which chemical assay is influential in human interactions is unclear. One approach to understanding how humans are influenced by biological odors has focussed on the information accessible through chemosignals, without specific knowledge about the chemical compounds involved. For instance, this approach has investigated the relationships between hedonicity of axillary odor and partner preferences based on genetic similarity (Wedekind et al., 1995), the effects of chemical secretions on the timing of the female menstrual cycle (McCintock, 1971,1998), and the relationship between personality and odor (Havlicek et al., 2005). An alternate approach has quantified the action of specific compounds on behavioral or psychological states, most notably a family of steroid compounds including estratetraenol, androstenol, androstenone, androstadienol and, particularly, androstadienone (review in Hays, 2003).

Androstadienone (4,16-androstadien-3-one) is an odorous compound found in axillary secretions (Brooksbank et al., 1972; Gower et al., 1994). Women exposed to androstadienone in the laboratory environment tend to experience positive affective modulation, including fewer feelings of boredom and frustration associated with the laboratory testing session, maintenance of positive moods despite exposure to negative stimuli, and increased feelings of focus (Bensafi et al., 2004a; Grosser et al., 2000; Jacob and McClintock, 2000; Jacob et al., 2001a; Lundström et al., 2003a;
Lundström and Olsson, 2005; Villemure and Bushnell, 2007). At the physiological level, androstadienone inhalation precedes measurable changes in endocrine state (Wyart et al., 2007) and autonomic activations (Bensafi et al., 2004a; Grosser et al., 2000; Jacob et al., 2001a; Monti-Bloch and Grosser, 1991) that may be specific to women (Boulkroune et al., 2007). Neurologically, female response to androstadienone extends beyond the olfactory system, activating areas of the brain associated with attention, social cognition, emotional processing, and sexual behavior (Gulyas et al., 2004; Jacob et al., 2001b; Savic et al., 2001; Savic et al., 2005). A functional consideration of these types of findings has led to the suggestion that androstadienone might have a sexual function, and be able to elicit behavioral responses in women towards men in such a manner as to facilitate partnership formation (Bensafi et al., 2004a; Hays, 2003; Lundström and Olsson, 2005), but so far specific evidence in support of this suggestion has been lacking (e.g. Lundström and Olsson, 2005).

Researchers have suggested that our understanding of androstadienone may be limited by the fact that its effects have only rarely been tested outside the laboratory (Jacob et al., 2002; Saxton et al., 2007). This is especially problematic because the effects of androstadienone appear to be highly context-dependent. For instance, two groups of researchers have found psychological influences of androstadienone when participants are tested by a male but not a female researcher, and suggest that the presence of a male may be necessary to provide sufficient ecological validity to elicit its specific effects (Jacob et al., 2001a; Lundström and Olsson, 2005). In addition, the effects of androstadienone can vary according to the nature of concurrent stimulus presentation (Bensafi et al., 2004a). To gain an accurate understanding of the role of androstadienone, and in particular whether it may influence male-female interactions,
we thus need to know its influences within a relevant social context (Jacob et al., 2002).

In light of this, we chose a speed-dating event as a suitable experimental context for testing the effects of androstadienone in relation to male-female interactions in the real world. Speed-dating allows single men and women to interact in pairs for very limited time periods. At the end of each interaction, men and women note covertly whether they would like to meet again. If both parties select the other, their contact details are exchanged by the organisers. Since the aim of participants is to evaluate and attract potential partners, speed-dating provides an ecologically valid and theoretically appropriate context to assess modulation of mate choice behaviors (Finkel et al., 2007).

Materials and methods

Participants

The study took place as three separate speed-dating events. Event 1 recruited 22 male and 25 female participants aged 19 – 25 (mean ± SD = 20± 1 yr) from among university undergraduates. To minimise the possibility that participants knew each other, female undergraduates were predominantly recruited from one university and male undergraduates from two separate but nearby universities. The event was held at the students’ union and organised by the authors. There was no charge for attendance, and participants received a voucher for a free drink at the end of the evening. The event was advertised as an experiment investigating the effects of odors, including human pheromones, on social judgments. Events 2 and 3 were organised through a private speed-dating agency for participants aged 30 – 45 and 21 – 35, respectively,
and took place in a local bar. Female experimental participants were recruited prior to the event by advertisements on the mailing list of the agency, and the usual fee was waived in exchange for their participation. The women were told that they were volunteering for an investigation into the science of attraction, and that they would be exposed to various odors during the evening. At Events 2 and 3, all male and a number of female speed-daters in attendance were not party to the experiment. Data were collected from 17 women aged 35 – 44 (mean ± SD = 39± 3 yrs) who interacted with 19 men in Event 2, and from 12 women aged 21 – 39 (mean ± SD = 32± 4 yrs) who interacted with 25 men in Event 3. All participants were instructed not to consume alcohol before or during the event, or to wear strongly scented products such as perfume or moisturisers.

**Procedure**

Participants provided informed consent. They used cotton wool pads to apply either water, clove oil (1% clove oil in propylene glycol), or 4,16-androstadien-3-one (250 µM concentration in the clove oil solution) in sufficient quantities to saturate the area of skin between mouth and nose, from an Eppendorf containing between approximately 0.5 and 1 ml of solution. The presentation method and concentrations were chosen to allow comparison with existing literature (e.g. Jacob and McClintock, 2000; Jacob et al., 2001b; Lundström et al., 2003a; Lundström and Olsson, 2005). Participants were not told which experimental condition they had been allocated to, nor the number of experimental conditions. Before the interactions began, the women answered a few questions (age, self-rated attractiveness, menstrual cycle dates, hormonal contraceptive usage, whether they smoked, whether they had consumed an alcoholic drink in the preceding couple of hours, description of the odor they were
given). Three of the women in the androstadienone condition (one from each Event) provided written descriptors of the solution (“urine, babies’ nappies”, “babies”, and “closses, musty/dirty”) that suggested that they may have consciously detected androstadienone; results reported below are qualitatively identical when the ratings given by these women are excluded.

On the basis of existing work showing measurable effects of androstadienone six minutes post-exposure and lasting at least two hours (Jacob and McClintock, 2000), we ensured a gap of at least 15 min between initial odorant application and the beginning of the male-female interactions. Event 1 interactions were completed a maximum of two hours and 15 min after first exposure. In Events 2 and 3, odorants were re-applied during a break approximately two hours after the initial application, and the interactions continued for approximately another hour following re-application. Women were seated at numbered tables, and men moved from table to table at regular intervals as prompted by the organisers. Participants had approximately three minutes to interact with and assess their partner. Female participants were provided with a score card on which they recorded a rating of each man’s attractiveness on a scale of 1 – 7, and indicated whether they would like to meet the man again (on the basis that if both participants selected the other, their contact details would be exchanged by the organisers). Photographs were taken of all female participants, and these were rated for attractiveness subsequent to the event by a panel of 15 (Event 1) or five (Events 2 and 3) male raters who had been chosen to be of similar age to those being rated.
Analysis

For the attractiveness rating scores, we discounted scores awarded to one man who left prematurely and so was rated by only five women; and all scores given by one woman who did not adhere to the rating system. A further 45 out of a possible 1190 ratings were excluded either because one or both of the participants indicated that they knew the other prior to the event, or because a rating had been omitted from the score card. A maximum of four scores awarded by any one woman, and a maximum of five scores awarded to any one man, were excluded in this way. These 45 omitted or excluded ratings were replaced by the average score given to the man in question, calculated from the remaining valid data. This is a conservative approach, reducing between-conditions variance.

We also calculated a man’s selection rate, namely, the proportion of women who selected a man as someone she might want to meet again, divided by the number of women who met that man. We excluded the man who left prematurely. Of the remaining men, two left somewhat early and so failed to meet four women each, and some potential meetings were missed (e.g. if a participant took a break; total omitted meetings $n = 15$ out of a possible 1173 meetings); the divisor in the calculation was adjusted as necessary.

Analyses were conducted with SPSS version 15.0. Mixed-model ANOVA with man ($n = 66$) as the unit of analysis and mean attractiveness rating or selection rate for each man calculated from all women in each of the three experimental Conditions (water, clove, androstadienone) as the three within-subject levels, following the analyses of Roberts et al (2005), and Wedekind et al (1995). Selection rate data were
non-normally distributed, but ANOVA is robust to deviations from normal distribution (Subrahmaniam et al., 1975). Greenhouse-Geisser correction was used in the mixed model analyses where data violated assumptions of sphericity. Event (whether the man was rated in Event 1, 2 or 3) was included as a between-subjects factor, although results are qualitatively identical if Event is excluded. Wilcoxon’s signed-ranks tests ($T$) were used in place of paired-samples t-tests ($t$), Kruskal-Wallis tests ($H$) used in place of one-way ANOVA, and Kendall’s tau correlations ($\tau$) in place of Pearson correlations, if data were non-normally distributed (Kolmogorov-Smirnov, $P<.05$).

**Results**

**Attractiveness ratings**

Experimental Condition (water, clove, androstadienone) had a significant effect on the rated attractiveness of the men ($F_{2,126} = 5.698$, $P = .004$). Men were rated significantly more attractive by women in the androstadienone condition compared with both women in the clove ($T = 606$, $P = .015$) and water ($T = 619.5$, $P = .003$) conditions. Ratings from the women in the clove and water conditions did not differ significantly ($T = 961.5$, $P = .468$).

The effect of Event on attractiveness rating bordered on significance ($F_{2,63} = 3.105$, $P = .052$): the trend was for men in Event 1 to be awarded higher ratings, although post-hoc comparisons of the ratings given at each Event revealed no significant differences (Games-Howell procedure: Event 1 and Event 2: $P = .1$; Event 1 and Event 3: $P = .12$; Event 2 and Event 3: $P = .99$).
The main effects of Condition and Event were modified by a significant interaction between Condition and Event (F\(_{4,126} = 4.892, P = .001\); Fig. 1), which was analysed further. In Event 1, Condition was significant (repeated-measures ANOVA of Event 1 as above, F\(_{2,42} = 20.69, P < .001\)). Men were given higher ratings by women exposed to androstadienone than women exposed to clove (t\(_{21} = .5763, P < .001\)) or water (t\(_{21} = 5.41, P < .001\)), but the ratings from the women in the water and clove conditions did not differ (t\(_{21} = .733, P = .472\)). In Event 2, Condition approached significance (F\(_{2,36} = 3.008, P = .062\)). The mean rating awarded in the androstadienone condition was higher than that awarded in the clove condition, which itself was higher than that awarded in the water condition, but the only statistically significant difference was between the androstadienone and water conditions (androstandiendone/clove (t\(_{18} = .988, P = .336\); androstadienone/water (t\(_{18} = 2.176, P = .043\); clove/water (t\(_{18} = 1.761, P = .095\)). In Event 3, Condition was not significant (F\(_{2,48} = .384, P = .683\); for completeness, figures are: androstadienone/clove T = 89, P = .564; androstadienone/water t\(_{24} = 1.091, P = .286\); clove/water t\(_{24} = .413, P = .683\).
In order to rule out other differences between the women in the three experimental conditions that may have been driving the differences in their attractiveness ratings, we compared the groups of women with respect to their self-rated attractiveness (Little et al., 2001), other-rated attractiveness (Penton-Voak et al., 2003), age, alcohol consumption and (in normally-cycling, non contraceptive users) menstrual cycle phase (Danel and Pawlowski, 2006; Gangestad et al., 2004). The women in the three Conditions did not differ significantly in any of these factors (self-rated attractiveness ($H_2 = .06, P = .970$); other-rated attractiveness ($F_2 = .522, P = .597$); age ($H_2 = .147, P$
= .929); whether they had had a drink in the preceding couple of hours \((H_2 = 2.034, P = .362)\). The number of normally-cycling women in the ‘fertile’ phase of the menstrual cycle, 14 – 20 days before predicted onset of next menses, bordered on significance \((H_2 = 5.681, P = .056)\), but while we might expect fertile-phase women to give higher attractiveness ratings (irrespective of chemical exposure; Danel and Pawlowski, 2006; Gangestad et al., 2004; but see Thorne et al., 2002 where no effect of menstrual cycle phase on attractiveness ratings was found), in fact the androstadienone Condition contained the smallest number of fertile-phase women (one of the 20 women exposed to androstadienone, two of 17 to clove, five of 15 to water).

Selection rates
Rated male physical attractiveness co-varied strongly with his chance of being selected for further meetings \((\tau = .741, P < .001; \text{Fig. 2})\).

![Figure 2](image)

Figure 2. The relationship between the proportion of women who selected a man for possible future meetings and mean attractiveness rating given to that man, by Condition. Each data point represents one man. The androstadienone condition was associated with higher attraction ratings \((P = .004)\) but not higher selection rates \((P = .495)\); attractiveness rating and selection rate covary positively (overall \(\tau = .741, P < .001\)).
There was no significant main effect of Condition on selection rates \((F_{1.8,115.0} = .681, P = .495)\). Selection rates differed significantly according to Event \((F_{2,63} = 3.164, P = .049)\), although post-hoc comparisons of the ratings given at each Event revealed no significant differences (Games-Howell procedure: Event 1 and Event 2: \(P = .096\); Event 1 and Event 3: \(P = .137\); Event 2 and Event 3: \(P = .997\)). These findings were modified by a significant interaction between Condition and Event \((F_{3.7,115.0} = 2.953, P = .027; \text{Fig.3})\). Separate repeated-measures analysis of each Event revealed a significant effect of Condition in Event 1 \((F_{2,42} = 5.136, P = .01)\); men were selected significantly less frequently by the women exposed to water than the women exposed to clove \((T=7, P=.005)\) or to androstadienone \((t_{21} = 2.973, P=.007)\), while clove and androstadienone conditions did not differ in selection rates \((t_{21} = .221, P=.827)\). There was no significant effect of Condition on selection rates in Event 2 \((F_{2,36} = 1.866, P = .169)\) or Event 3 \((F_{1.6,38.6} = 1.623, P = .213)\).
Discussion

We investigated the effects of androstadienone exposure on women’s attractiveness ratings, and selection, of men, at three separate speed-dating events, in a between-subjects design. Speed-dating was chosen as a suitable arena to investigate a chemical with a posited sexual signalling function. Women applied either androstadienone mixed in a solution that smelt of clove oil, clove oil alone, or water, to the upper lip, enabling passive inhalation of the solution throughout the evening. The three experimental conditions allowed us to parse the effects of an odor from the specific effects of androstadienone (Hays, 2003; Pause, 2004).
We found some evidence that androstadienone exposure can enhance ratings of men’s attractiveness. Men were rated more attractive overall by women exposed to androstadienone than by women exposed to clove oil or water, and the groups of women given the three different solutions did not differ in other factors known to affect ratings of attractiveness (i.e. self-rated attractiveness, other-rated attractiveness, age, menstrual cycle phase, alcohol consumption).

However, the main effect of androstadienone exposure was modified by a significant interaction with the event (i.e. at which of the three separate speed-dating events the data were collected). The most compelling evidence for the effects of androstadienone came from first event, with some confirmatory evidence from the second event, and no demonstrable effect of androstadienone at the third. The reasons for this interaction are unclear. Sampling effects could be at issue: the first event contained the most, and the third the fewest, female participants. The effects of androstadienone appear to be amenable to context, and it is likewise possible that differences between the speed-dating events were influential. The first event, organised by the authors, was most carefully controlled, and participants may have been more motivated; certainly, at the first event there was a trend for overall ratings of attractiveness and selection rates to be higher. Alternatively, a greater proportion of the women in the first event may have been sensitive to the effects of androstadienone. The composition of the people in the studies may also play an important role. The first event contained the youngest sample, where physical traits may be relatively more important than, for example, financial status, compared to older samples, and this could increase the chances that odor cues might be important. Results may also be prone to variation amongst the attractiveness of the people participating in each event.
Although exposure to sub-threshold concentrations of non-odorous sweat (but not synthetic, odorous sweat, Demattè et al., 2007) has been shown to enhance women’s ratings of men’s attractiveness (Thorne et al., 2002), a previous experiment, in which the effect of androstadienone on ratings of facial photographs was measured, detected no significant response (Lundström and Olsson, 2005). If androstadienone can modulate social perceptions, it is possible that ecologically valid accompanying cues, such as a man’s presence, are necessary for this effect to emerge (Jacob et al., 2001a; Lundström and Olsson, 2005; Saxton et al., 2007).

The finding that androstadienone may enhance women’s ratings of men’s attractiveness is consistent with the idea that it may constitute a sexual signal that conveys information about male mate quality. The finding that women show concordant strength of preference for male facial masculinity and the odor of androstadienone has been interpreted as indirect evidence that androstadienone may index male mate quality (Cornwell et al., 2004), and in male boars, production levels of a related chemical, a boar pheromone called androstenone, correlate positively with social rank and aggression levels (Giersing et al., 2000).

Although we assume that our results are due to an effect of androstadienone on the women, it is not inconceivable that the men were also behaving differently in its presence, despite their greater distance to the source of the odor. Certainly, male responses to androstadienone have been noted previously, and these often differ in direction from the female response (Bensafi et al., 2004b; Chopra et al., 2008; Jacob and McClintock, 2000).
An understanding of the effects of androstadienone is still far from complete, and this required us to make a number of assumptions in our experimental design. Firstly, although we used an experimental context that was more ecologically valid than a standard laboratory test, the method of application of androstadienone, and possibly also the dosage, remains somewhat artificial (Saxton et al., 2007), and may be consciously detectable by a minority of the population (Lundström et al., 2003b). An alternative design, which could solve this shortcoming, would be to apply the odor stimuli to the men, rather than to the women; and to use sub-threshold concentrations without the clove oil admixture. Although the use of clove oil as a masking odor allows for comparison with previous literature, its usage has been criticised on the basis that the experimental solution then constitutes a complex mixture (Lundström et al., 2003a), and also that it has a number of biological effects including anesthesia (Chaieb et al., 2007). Related to these problems, it is as yet unclear whether androstadienone can be expected to have any effect in naturalistic settings other than in very intimate dyadic encounters, or if it does, how a man might solve the problem of influencing a woman’s response to him without influencing her response to all other men in the vicinity.

Our results represent a novel demonstration of the modulation of mate choice behavior. Yet they are also consistent with a range of findings which may constitute the proximate mechanisms of the modulation: enhancement of female positive mood (Bensafi et al., 2004a; Bensafi et al., 2004b; Jacob and McClintock, 2000; Jacob et al., 2001a; Lundström and Olsson, 2005; Villemure and Bushnell, 2007); and increased ease-of-processing of facial characteristics in the presence of related cues such as bodily odors (Kovacs et al., 2004; Rowe, 1999). While it is possible that
attractiveness modulation is a mere corollary of these previously noted effects, it could as easily be argued that modulation of reproductive behavior is the primary function. Our studies are highly suggestive that the presence of androstadienone can increase a woman’s attraction to a man in a mate-choice context, though the strength of this effect and the exact context in which it applies remain questions for future research.

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