Smelling 3β-Androstenol Dampens the Stress Induced Rise in Cortisol in Young Females

3β-Androstenolün Sağlıklı Genç Kadınlarda Stresle Tetiklenen Kortizol Artışı Üzerindeki Baskılayıcı Etkisi

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ABSTRACT Objective: The present study was designed to investigate the role of 3β-androstenol (3β-AND) in the modulation of stress induced serum cortisol levels and alterations in the mood of young adult females. **Methods:** A total of 79 healthy females (mean age= 21.24 ± 2.26) were included in the study composed of control (n= 9), vehicle (n= 9), 3β-AND (n= 12), stress (n= 23), 3β-AND-stress (n= 21) and vehicle-stress (n= 5) groups to determine respective levels of serum cortisol and estradiol and the mood scores. Menstrual phase distribution was similar between experimental groups (p> 0.05). Effects on mood were measured using a 16-item mood test. Public speaking method including speaking in front of the audience was applied which was known to be an efficient laboratory stressor leading to significant cardiovascular, endocrine and mood alterations in the individuals without any real threat of physical danger or harm. **Results:** Higher scores for sexual arousal (p< 0.01 for each) and positive mood were obtained in 3β-AND group when compared to stress and 3β-AND-stress groups. Highest serum cortisol levels (μg/dL) were obtained for the stress group (p< 0.001). Neither the mood scores nor the serum levels of cortisol were found to differ with respect to menstrual cycle (p> 0.05). 3β-AND was rated as a neutral substance by 45% of the subjects. All of the subjects (n= 10, p< 0.001) indicating an intense sensation about 3β-AND were in the pre-ovulatory phase of the menstrual cycle. **Conclusion:** In conclusion 3β-AND whether perceived as a neutral substance or an odorous compound, seems to dampen the stress induced rise in cortisol effectively in young healthy females.

Key Words: Pheromones; stress, physiological; menstrual cycle

ÖZET Amaç: Bu çalışma 3β-androstenol (3β-AND) ün genç kadınlarda stresle indüklenen serum kortizol düzeyi ve duygudurum değişikliklerine etkisini değerlendirmek amacıyla tasarlandı. **Gereç ve Yöntemler:** Gönüllülük esasına göre çalışmaya dahil edilen toplam 79 sağlıklı genç kadın (ortalama yaş= 21.24 ± 2.26); kontrol (n= 9), taşıyıcı (n= 9), 3β-AND (n= 12), stres (n= 23), 3β-AND-stres (n= 21) ve taşıyıcı-stres (n= 5) olmak üzere deney gruplarına ayrılarak serum kortizol, estradiol ve mod skorları açısından değerlendirildi. Grupların menstrüel siklus evresi dağılımları açısından homojen olduğu tespit edildi (p> 0.05). Duygudurum değerlendirmesi 16 maddelik mod değerlendirme testi kullanılarak yapıldı. Psikolojik stresör olarak, kişi üzerinde herhangi fiziksel bir rahatsızlık veya tehlike oluşturmaksızın belirgin kardiyovasküler, endokrin ve duygudurum değişikliklerine yol açtığı bilinen topluluk önünde konuşma yöntemi uygulandı. **Bulgular:** 3β-AND grubunda elde edilen cinsel uyarılma (p< 0.01) ve pozitif mod (p< 0.01) skorları; stres ve 3β-AND-stres gruplarına göre anlamlı şekilde daha yüksek olarak tespit edildi. En yüksek serum kortizol düzeyleri (μg/dL) stres grubunda (p< 0.001) saptandı. Duygudurum skorları ve serum kortizol düzeylerinin menstrüel siklusun farklı evrelerinde benzer değerlerde olduğu bulundu (p> 0.05). 3β-AND deneklerin %45'i tarafından nötral bir madde olarak algılanırken, maddeyi yoğun olarak algılayan tüm deneklerin (n= 10, p< 0.001) ovülasyon öncesi dönemde oldukları saptandı. **Sonuç:** 3β-AND nötral veya kokulu bir madde olarak algılanmasından bağımsız olarak, stresle indüklenen kortizol artışını genç kadınlarda etkin şekilde baskılayabilmektedir.

Anahtar Kelimeler: Feromonlar; stres; menstrüel siklus

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Pheromones are defined as airborne chemical signals released into the environment by an individual and affect the physiology and behavior of other members of the same species.[1]

Being putative pheromones; nonandrogenic 16-androstenes including androstenones and androstenoles have been studied so far in terms of their influence on the psychological state and behavior.[2] Among them 5α-androstenone, androstadienone, androstadienol, 3α-androstenol and the 3β-androstenol (3β-AND) were detected to contribute to the specific odour of the human axillae.[3]

Androstenones were shown to have dose dependent and gender-specific effects on both mood and autonomic nervous system responses.[4-6] However, as they quickly convert to 3α-androstenoles upon air exposure, some of their behavioral effects have been attributed in part to the 3α-androstenoles.[3]

Compared to studies conducted with other pheromone-like compounds, limited data exist on androstenoles including the influence of 3α-androstenol (5α-androst-16-en-3α-ol), detectable in the female axillary secretions, on the menstrual synchrony among females.[7]

Moreover, the present data did not directly address the pathway by which androstenones modulated the autonomic function and the mood. Vomeronasal organ (VNO) which represents the way of chemical communication between conspecifics in non-human mammals was also suggested to have a role in the mediation of the androstenone effect in humans.[5,8,9] Other studies, however, have questioned the presence of a functional human VNO indicating the role of direct absorption into the circulation rather than acting through chemical sensing (either VNO or the main olfactory system) per se.[10,11]

Signaling effects and the ability of body odor compounds to directly alter human mood or behaviour were investigated using androgen steroids; however, the results are conflicting.[12] It remains unclear whether androgen steroids strengthen or reduce positive emotions or do not affect female behaviour.[4,13,14]

Since conscious odors were reported to dampen the cortisol response to a stressor, the present study was designed to investigate the influence of smelling 3β-AND (5α-androst-16-en-3β-ol) on the stress induced alterations in the mood scores and the serum cortisol levels.[15,16]

MATERIAL AND METHODS

SUBJECT POPULATION

A total of 79 healthy female undergraduate students aging between 18-25 years and having a menstrual cycle of 28-30 days were recruited for the study. Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the local ethics committee (4.10.2005-10.17.2005-Protocol No. 191).

Following 3 months follow up of volunteers for the menstrual cycle patterns; they were re-evaluated according to the inclusion and exclusion criteria of the study presented in Table 1.

Subjects were divided into three groups, composed of menstrual bleeding (1-2nd day of the cycle, n= 27), pre-ovulatory LH surge (postmenstrual 14 ±

TABLE 1: Inclusion and exclusion criteria for the study.

Inclusion criteria
• To have regular menstrual cycle with a rhythm of 28-32 days
• No past history of endocrine, cardiovascular, neurological and psychiatric diseases
• No acute or chronic disorder that may interfere with olfactory function such as URTI, allergic rhinitis, asthma
• No history of or tendency to be faint during blood collection
Exclusion criteria
• Will to leave
• Use of an oral contraceptive medication
• Over reactivity during basal measurements
• Acute development of endocrine, cardiovascular, neurological, psychiatric or respiratory disease
• Sudden changes in the menstrual cycle pattern
• Development of previously unknown and unpredictable discomfort during any of the experimental procedures.

2. day; n= 26), and the midluteal (post-ovulatory 8 ± 1. day; n= 26) phases of the menstrual cycle. Pre-ovulatory LH surge was confirmed by urine test detecting the LH peak (Rapidan Ovula LH®, Turk-Lab, Izmir, Turkey). Serum estradiol levels were measured to confirm the appropriate menstrual phase of the subjects.

Each menstrual phase was equally represented in the experimental groups composed of stress (n= 23), 3β-AND (n= 12), 3β-AND-stress (n= 21), vehicle (sesame oil; n= 9), vehicle-stress (n= 5) and control groups (no treatment; n= 9).

Following baseline period, first mood test was applied to the subjects. Experimental procedures concerning 3β-AND and stress administration were performed before the second mood test which was applied after the resting period. Experimental timeline was presented in Table 2.

MOOD SCALE

Effects on mood were measured using a 16-item mood test. Subjects rated how strongly they were experiencing each of 16 different moods on a 9-point scale with 1 corresponding to "not at all" and 9 corresponding to "very strongly". This mood test was devised to tap into mood rather than more transient emotional feelings.[17,18] It is well validated and consists of the following variables: afraid, amused, angry, annoyed, anxious, bored, calm, confident, content, contemptuous, disgusted, embarrassed, happy, interested, sad, and stressed. "Sexually aroused" was also added to this test and used as a descriptor as described previously in the literature.[19]

COMPOUNDS

10 mg (0.00125 M) of 3β-AND (5α-androst-16-en-3β-ol; Sigma-A7386) was dissolved in 30 mL of sesame oil which was defined as a clear, light yellow to almost colourless and odourless liquid in the product specification. Thirty milliliter of sesame oil served as a vehicle. Both solutions were put into identical 60 mL opaque jars (4.5 cm in diameter at the opening; 8 cm high) as defined previously.[19] Appropriate experimental jar presented by the researcher was sniffed by the subjects as long as 60 seconds. Subjects were asked to identify the compound in terms of perceived intensity, familiarity, pleasantness or neutrality. Moreover, to assess possible trigeminal nerve effects, participants were asked to rate any sensations of itching or burning.

TABLE 2: Experimental design.

Baseline period	Mood test-1	(3β-AND) Stress (3β-AND)-stress Control/vehicle Vehicle-stress	Resting period	Mood test-2 Blood collection
5 minutes	5 minutes	5 minutes	10 minutes	5 minutes

PUBLIC SPEAKING

Public speaking was considered as an efficient laboratory stressor leading to significant cardiovascular, endocrine and mood alterations in the individuals without any real threat of physical danger or harm.[20] The applied task in the present study was speaking freely about the topic chosen by the subject randomly among the items provided in a list, as long as 3 minutes in front of the audience.

HORMONE LEVELS

At the end of the experiment, blood was drawn from each subject for the measurements of cortisol (mg/dL) and estradiol (E2; pg/mL) levels. Time of the blood sampling (10:30-11:00 a.m) was also recorded for the apropriate measurement for the hormone levels. Following centrifuge serum samples were stored at -20°C until the biochemical analysis which was accomplished by referans lab (Istanbul, Turkey).

STATISTICAL ANALYSIS

Database was transferred to SPSS (Statistical Package for Social Sciences). Statistical analysis of the data was made using SPSS 13.0 version. Chi-square (χ^2) or Fisher's test for independent categorical variables; Student's t-test and analysis of variance (ANOVA) for parametric and Mann-Whitney U and Kruskal-Wallis tests for non-parametric variables were used for the statistical evaluation. Post Hoc evaluation of data was made via Tukey or Tamhane's test. Data were expressed as "mean ±

standard error of mean (SEM)" and percent (%) where appropriate. p< 0.05 was considered statistically significant.

RESULTS

A total of 79 females (mean age= 21.24 ± 2.26) who were distributed homogeneously according to menstrual phase and experimental groups; were included in the study (Table 3). Mean age values were similar among experimental groups (21.17 ± 0.48 for the stress group; 22.0 ± 0.46 for the 3β-AND-stress group; 21.41 ± 0.73 for the 3β-AND group; 20.22 ± 0.43 for the control group; 20.33 ± 0.74 for the vehicle group and 21.40 ± 1.28 for the vehicle-stress group; p> 0.05)

Considering mood scores; the highest rating scores for sexual arousal were obtained in 3β-AND group (3.08 ± 0.85) when compared control (0.88 ± 0.58; p< 0.05), vehicle (0.9 ± 0.68; p< 0.05), stress (-0.86 ± 0.4; p< 0.001), 3β-AND-stress (-0.38 ± 0.4; p< 0.001) and vehicle-stress (-0.66 ± 0.83; p< 0.001) groups (Figure 1). Highest scores for the positive mood were determined in the 3β-AND group (7.08 ± 1.2) when compared to control (1.66 ± 0.47; p< 0.05), vehicle (1.0 ± 0.43; p<0.05), stress (-0.9 ± 1.3; p< 0.001), 3β-AND-stress (0.6 ± 1.5; p< 0.01) and vehicle-stress (-1.2 ± 1.5; p< 0.01) groups (Figure 2).

Highest serum cortisol levels (μg/dL) were obtained for the Stress group (15.16 ± 0.95 μg/dL) when compared to control (7.48 ± 0.44 μg/dL; p< 0.001), vehicle (9.5 ± 0.76 μg/dL; p< 0.01), 3β-AND (8.68 ± 0.54 μg/dL; p<0.001), 3β-AND-Stress (9.46 ± 0.81 μg/dL; p< 0.001) groups (Figure 3).

Serum estradiol levels were significantly lower in the menstrual bleeding phase (43.19 ± 10.05 pg/mL) when compared to midluteal (154.2 ± 14.29 pg/mL, p< 0.01) and pre-ovulatory (109.5 ± 11.7 pg/mL, p< 0.05) levels (Table 2). Neither the mood scores nor the serum levels of cortisol were found to differ with respect to menstrual cycle (p> 0.05; Table 3).

3β-AND was rated as a neutral substance by 45% of the subjects. Among the subjects (55%) perceived a conscious odor upon sniffing the substance, only 8 (24%) rated the compound familiar. In addition to this, all of the subjects (n= 10, p< 0.001) indicating an intense sensation about 3β-AND were in the pre-ovulatory phase of the menstrual

TABLE 3: The effects of 3β-AND on serum hormone levels, smell quality and mood scores with respect to menstrual phase.

		Menstrual phase		
		Mid-luteal	**Bleeding**	**Pre-ovulatory**
		(n= 26)	**(n= 27)**	**(n= 26)**
Experimental groups	Control (n= 9)	3 (33.3%)	3 (33.3%)	3 (33.3%)
	Vehicle (n= 9)	3 (33.3%)	3 (33.3%)	3 (33.3%)
	Stress (n= 23)	7 (30.4%)	9 (39.1%)	7 (30.4%)
	3β-AND (n= 12)	5 (41.7%)	4 (33.3%)	3 (25%)
	3β-AND-stress (n= 21)	7 (33.3%)	6 (28.6%)	8 (38.1%)
	Vehicle-stress (n= 5)	1 (20%)	2 (40%)	2 (40%)
Serum estradiol levels (pg/mL)		154.2 ± 14.29**	43.19 ± 10	109.5 ± 11.7*
Serum cortisol levels (μg/dL)		11.97 ± 1.1	10.47 ± 0.8	11 ± 0.7
Smell of 3β-AND+	Odorous (n= 18)			
	Intense (n= 10)	0 (0%)	0 (0%)	10 (100%)
	Familiar (n= 8)	4 (50.0%)	4 (50%)	0 (0%)
	Neutral (n= 15)	8 (53.3%)	6 (40%)	1 (6.7%)
Positive mood scores		1.0 ± 1.33	1.07 ± 1.17	1.19 ± 1.01
Sexual arousal		0.42 ± 0.4	0.18 ± 0.4	0.30 ± 0.6

Data were shown as n (%) or mean ± SEM. + p< 0.001 (χ^2);

* p< 0.05, and ** p< 0.01; compared to estradiol levels of subjects in the bleeding phase of menstrual cycle.

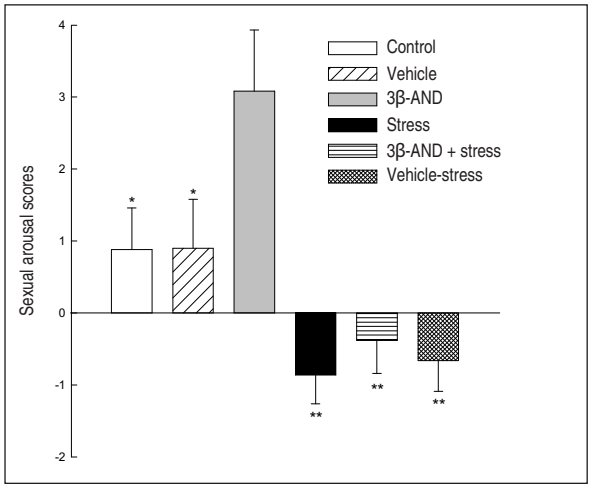

FIGURE 1: Sexual arousal scores in subjects (n= 79) with respect to experimental groups composed of control (n= 9), vehicle (n= 9), Stress (n= 23), 3β-AND (n= 12), 3β-AND-stress (n= 21) and vehicle-stress (n= 5) groups. 3β-AND: androstenol.

* p< 0.01, ** p< 0.001; compared to androstenol group.

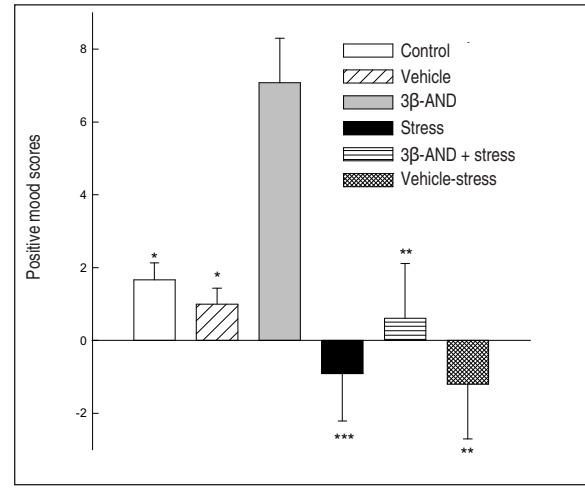

FIGURE 2: Positive mood scores in subjects (n= 79) with respect to experimental groups composed of control (n= 9), vehicle (n= 9), stress (n= 23), 3β-AND (n= 12), 3β-AND-stress (n= 21) and veh-stress (n= 5) groups. 3β-AND: androstenol.

*p< 0.05, ** p< 0.01 and ** p< 0.001; compared to androstenol group.

cycle. None of the subjects rated the compound pleasant or sensations of itching or burning (Table 3). Subjective rating of 3β-AND as a neutral or odorous substance was found to have similar influence on stress induced cortisol rise and mood and arousal scores (Table 4).

DISCUSSION

Higher release of the stress hormone cortisol in our subjects under laboratory stress confirms the aversive and stressful nature of public speaking task initiating the release of cortisol through the hypothalamus-pituitary-adrenal (HPA) axis.[21]

In contrast to previously reported increase in salivary level of the cortisol in women after smelling pure androstadienone,[22] 3β-AND was not associated with an alteration in the serum cortisol levels unless coupled with the laboratory stressor in the present study.

In agreement with the past studies concerning the effects of androstenones on the reduction in nervousness, tension, other negative feelings and increase in positive mood, androstenol was also found to have mood elevating effect in our subjects.[4,5,19] Provided that not coupled with the stressor, 3β-AND alone seems to induce higher mood and sexual arousal.

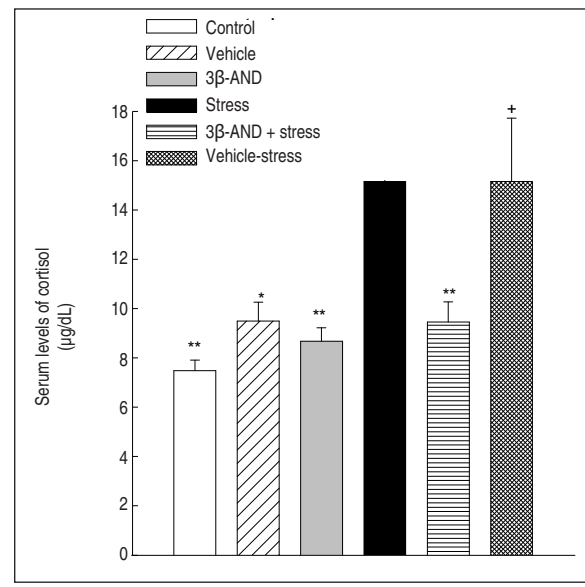

FIGURE 3: Serum cortisol levels in subjects (n= 79) with respect to experimental groups composed of control (n= 9), vehicle (n= 9), stress (n= 23), 3β-AND (n= 12), 3β-AND-stress (n= 21) and veh-stress (n= 5) groups. 3β-AND: androstenol.

* p< 0.01 and ** p< 0.001; compared to stress group

+ p< 0.05; compared to 3β-AND-Stress group

Rating of 3β-AND by approximately half of our subjects as a neutral substance was compatible with the previously reported specific anosmia rates (2-75%) for androstenes in adults.[23] Besides 3β-AND influenced the stress induced cortisol levels

TABLE 4: Mood scores and serum hormone levels according to rating feature of 3β-AND.

	Odorous	Neutral
Positive mood score	2.38 ± 1.48	3.66 ± 1.94
Sexual arousal	0.94 ± 0.69	0.8 ± 0.78
Serum cortisol level (μg/dL)	9.44 ± 0.59 μg/dL	8.86 ± 0.99 μg/dL
Serum estradiol level (pg/mL)	102.89 ± 14.12	87.44 ± 16.72

Data were shown as mean ± SEM. p> 0.05.

in the same range whilst the different sensational routes including neutral or odorous rating of the compound. In this manner, 3β- AND seems to modulate the conscious assessment of psychological state as well as endocrine responses to that interaction without such necessity of being recognized or classified as an odor.

Alteration of the hormonal patterns during different phases of the menstrual cycle has been known to affect several psycho-physiological processes associated with emotional status and stress reactivity.[24,25] Higher level of estrogen in the ovulatory phase was proposed to produce general arousal via central effects (e.g., corticotropin releasing factor) on the HPA and sympatho-adrenomedullary (SAM) systems.[26-28] Ovulation specific intense perception of 3β-AND may have a role in the stimulation of the fertility as cortisol is known to affect the reproductive health negatively contributing to low sex drive and infertility.[29] In that manner, cortisol lowering effect of 3β-androstenol under stresfull condititons may have a key role in the reproductive success.

There is wide agreement that mate choice adaptations in females have evolved to select males who will confer benefits on offspring, through direct and indirect routes.[30] Women's preferences for indicators of good genes including were stated to peak near ovulation.[31] Sensitivity to putative pheromones was reported to be due to increased olfactory sensitivity if menstrual cycle pattern was considered. Women were stated to perceive olfactory stimuli with a higher sensitivity during the ovulatory phase and describe odors differentially during this period.[32] Ovulation specific increase in intensity of odor perception concerning 3β-AND in our study correlates with this statement which has reproductive implications.

In conclusion, ovulation specific increase in perceptional sensitivity of 3β-AND seems to indicate the priority of the reproductive success by reversal of stress induced cortisol levels. Since its enhancing effects on mood and the sexual arousal of subjects fail to counteract the stress related disturbance in these parameters, possible modulatory role of non-androgenic 16-androstenes in the interaction between hypothalamo-pituitary-adrenal axis and reproductive success needs further and more detailed evaluation considering determination of basal cortisol levels in larger populations. Elucidation of such sensory mechanisms associated with the act of putative pheromones may help the possible use of human chemical signals as natural endocrine therapeutics.

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