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Efficient sweat reduction of three different antiperspirant application forms during stress-induced sweating

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Synopsis

OBJECTIVES: Stress sweating can occur in everyday situations independently of thermally-induced perspiration. It is triggered by emotionally challenging situations and leads to underarm wetness and a characteristic unpleasant malodor. In this study, we aimed to determine the long-term efficacy of three unperfumed antiperspirant (AP) formulas for different application forms (roll-on, stick, aerosol) against stress-induced sweating and malodor formation.

METHODS: We utilized the widely accepted Trier Social Stress Test (TSST) to induce psychosocial stress in female and male volunteers (18 – 40 years) and determined physiological stress parameters. To additionally assess the efficacy of the test AP roll-on against thermally-induced sweating, a hot room study was performed.

RESULTS: Increasing heart rates and an augmentation of saliva cortisol levels during the TSST indicated a substantial stress reaction which was paralleled by a pronounced sweat production in the untreated axillae of both males and females. Forty-eight hours after application, all three test APs significantly decreased the amount of sweat in the treated axillae independent of gender. With respect to AP effects on malodor production, trained sniffers assessed sweat samples collected during the TSST from the untreated axillae as significantly more malodorous than comparable samples from the AP-treated axillae. Also, independent of gender the test AP roll-on significantly decreased the thermally-induced sweat in the AP-treated axilla.

CONCLUSION: We show for the first time a highly effective reduction of emotionally-induced axillary sweating and malodor production for three different application forms 48 h after the last product use. The specially developed roll-on, stick, and aerosol AP provide long-term protection against stress-induced sweat which is of high relevance in everyday life.

Résumé

OBJECTIF: La transpiration de stress peut se produire dans des situations quotidiennes indépendamment de la transpiration induite par la chaleur. Elle est déclenchée par des situations de charge émotionnelle et conduit à la moiteur sous les bras et à une mauvaise odeur désagréable caractéristique.

MÉTHODE: Dans cette étude, nous avons cherché à déterminer l'efficacité à long terme de trois formules anti-transpirantes sans parfum (AP) pour les différents formes d'application (roll-on, bâton, aérosol) contre la transpiration induite par le stress et la formation de mauvaises odeurs. Nous avons utilisé le Social Trier Stress Test (TSST) largement acceptée pour induire un stress psychosocial chez les femmes et hommes (18 - 40 ans) aux paramètres physiologiques de stress déterminés. Pour évaluer en plus l'efficacité du produit AP roll-on contre la transpiration induite thermiquement, une étude dans la chambre chaude a été effectuée.

RÉSULTATS: Le rythme cardiaque qui augmente et une augmentation du taux de cortisol salivaire au cours de la TSST indiquent une réaction de stress importante qui est accompagnée d'une production de sueur prononcée dans les aisselles non traitée de personnes féminines et masculines. Quarante-huit heures après l'application, les trois AP testés diminuent de manière significative la quantité de sueur sous les aisselles traitées, indépendamment du sexe. En ce qui concerne les effets sur la production de mauvaises odeurs, des renifleurs entraînés ont évalué des échantillons de sueur recueillies au cours de la TSST des aisselles non traitées comme significativement plus malodorant que des échantillons comparables de la aisselles traitées. En outre, indépendamment du sexe, le produit AP roll-on testé diminue de manière significative la sueur induite thermiquement dans l'aisselle AP-traitée.

CONCLUSION: Nous montrons pour la première fois une réduction très efficace de la transpiration axillaire émotionnellement induite et la production de mauvaises odeurs pour trois formes d'applications différentes 48 h après la dernière utilisation du produit. Les roll-on, bâton et aérosol AP spécialement développés fournissent une protection à long terme contre la transpiration induite par le stress, qui est de grande importance dans la vie quotidienne.

Introduction

Stress signals evoke neuroendocrine responses in humans stimulating cardiovascular, metabolic and endocrine processes that enable the individual to cope with stress. One physiological response triggered in a stressful situation is the secretion of sweat, which is generated by eccrine and apocrine sweat glands. In an emotionally challenging situation, both eccrine and apocrine sweat glands are being activated [1, 2]. Eccrine sweat glands are present over the entire body surface. In contrast, apocrine sweat glands are found only in the axillary, mammary, perineal and genital regions of the human body. It has long been thought that emotion-induced sweating is characterized by an enhanced sweat production mostly on glabrous skin surfaces and in the axilla [1–4]. However, stress-evoked eccrine sweating may be a ubiquitous phenomenon, which

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is at room temperature only apparent on palms, soles and axillae [5, 6] due to the higher density of sweat glands in these areas.

Eccrine sweat glands secrete a watery fluid and are mainly involved in thermoregulation. In contrast, apocrine sweat glands are stimulated by stress, fear or mental tension and do not become active until puberty [7–9]. In the axilla, both types of sweat glands are present. Eccrine glands keep the axilla moist through thermally and emotionally induced secretions. Human body odour is to a large extent determined by secretions originating from apocrine sweat glands. Freshly produced apocrine sweat is milky, odourless and contains proteins, lipids and steroids [10]. Odouriferous compounds are generated from non-smelling precursors by normal cutaneous bacterial activity [11], for example by removal of hydrophilic residues, leaving low-molecular-weight volatile molecules. The special cavern-like shape and the moist environment of the axilla provide excellent growth conditions for bacteria. Microbial biotransformation is performed by four major groups of axillary bacteria, such as staphylococci, aerobic coryneforms, propionibacteria and micrococci [12–17]. Strong malodour formation is mainly attributed to the activity of corynebacteria [18]. Interestingly, the types of bacteria colonizing the axilla can vary considerably between individuals as well as between genders. Slight differences have been described even between the right and left axilla of one volunteer [19]. In the axilla, the emotionally triggered sweat secretion is even more pronounced with increasing temperatures [20].

By most societies, excessive sweating and the subsequent malodour formation are perceived as offensive. As a result, self-confidence and social relationships can be influenced by undesired body odour [21]. Out of these reasons, in many populations worldwide, antiperspirants (AP) and deodorants (Deo) are used on a regular basis to reduce and control sweating and axillary malodour. As today's societies are becoming more and more fast-moving, personal requirements increase and lead to augmented individual stress levels. Against this background, special APs protecting reliably against stress-induced sweating and malodour are needed. In this study, we induced emotional sweating to study the long-term efficacy of three Deo/AP formulas for different application forms (roll-on, stick, aerosol) against stress-induced sweating and axillary malodour.

Materials and methods

In vivo studies

Two different *in vivo* studies were conducted. Study I: three formulas for different application forms (roll-on, stick, aerosol) were tested against stress-induced sweating. Study II: the efficacy of the roll-on AP against thermally induced sweating was assessed.

Healthy, non-smoking males and females were included in both study types. Volunteers had to be willing to shave their axillae prior to the first test product application and then refrain from shaving throughout the study. Exclusion criteria for study participation were pregnancy, lactation, intolerance to cosmetic products, known allergies to AP-products containing aluminium complexes, hyperhidrosis and former Trier Social Stress Test (TSST) participation (if applicable). All participants provided written informed consent.

To standardize axillary condition prior to either study start, all volunteers took part in a conditioning phase (Study I: 14 days; study II: 21 days). During this period, a perfume-free washing liquid (pH 5.5; Baktolin® Basic Pure, Bode Chemie, Hamburg) for washing of armpits and a commercially available aluminium-free deodorant (Unity® 8 × 4, Beiersdorf AG, Hamburg, Germany) were supplied by Beiersdorf AG for use at home. Two (Study I) or 7 days (Study II) prior to the study start, volunteers were asked to continue using the washing lotion but to refrain from utilizing any AP or deodorant (also the wash-out deodorant) as well as perfume and other leave-on products on their axillae. With respect to Study II, the use of hairspray was prohibited. Volunteers were also required to refrain from eating garlic or highly seasoned food. Visits to saunas and swimming pools as well as intensive sports were prohibited for 1 day prior to each scheduled application and measurement.

Study I: Stress-induced sweating

The study determining the efficacy of the test APs against stress-induced sweating was approved by the International Medical and Dental Ethics Commission Freiburg (Germany; study numbers: B11-2012 and B12-2012) and was carried out in compliance with the Declaration of Helsinki and ICH/GCP. The study was performed at the study facilities of DAaCRO GmbH & Co KG (Trier, Germany) and conducted based on a randomized, open-label design.

Test products

AP roll-on. The test AP roll-on contained aqua, aluminium chlorohydrate (ACH), PPG-15 stearyl ether, steareth-2, steareth-21, aluminium sesquichlorohydrate (ASCH), zinc citrate, Persea gratissima oil and trisodium EDTA. On 4 consecutive days, 500 mg test AP per axilla was applied with a standard roll-on reflecting the amount that a typical individual would utilize under normal use conditions.

AP stick. The test AP stick comprised stearyl alcohol, PPG-14 butyl ether, aluminium zirconium tetrachlorohydrex gly (AZG), cyclomethicone, caprylic/capric triglyceride, paraffinum liquidum, talc, hydrogenated castor oil, ASCH, glyceryl stearate SE, zinc citrate, Persea gratissima oil, octyldodecanol and BHT. On 5 consecutive days, 500 mg test AP was applied per axilla.

AP aerosol. For the study, two different gender-specific AP aerosols were used. The *female product* contained butane, isobutane, propane, ACH, cyclomethicone, isopropyl palmitate, ASCH, zinc citrate, Persea gratissima oil, octyldodecanol, disteardimonium hectorite, dimethicone, propylene carbonate and dimethiconol.

The *male product* contained butane, isobutane, propane, ACH, cyclomethicone, isopropyl palmitate, ASCH, zinc citrate, menthoxypropanediol, Persea gratissima oil, octyldodecanol, disteardimonium hectorite, dimethicone, propylene carbonate and dimethiconol. On 5 consecutive days, a two-second spray was applied per axilla. This is equivalent to a weight loss of the can (including propellant gas) of approximately 2.0 g for the female formula and 2.5 g of the male formula.

Participants

Initially, 30 volunteers (15 women/15 men; age: 18–40 years) were recruited to apply the test AP roll-on. Likewise, 30 volunteers (15 women/15 men; age: 20–39 years) were included into the AP stick test group. A total of 15 women volunteers were recruited for the female-specific test AP aerosol (age: 18–36 years) and 20 men volunteers (age: 19–38 years) for the male-specific test AP aerosol. Volunteers' state-of-health was checked by a medical interview. All subjects entered a 14-day conditioning phase.

After having completed the wash-out period, volunteers visited DAaCRO's study facilities for repeated standardized application of the assigned test AP. Each time, the test AP was applied to one axilla according to a randomization scheme by qualified DAaCRO personnel. The contralateral axilla was left untreated. To determine the quantity of applied AP, the weight of test product containers was measured gravimetrically using an electronic precision balance (Soehnle professional standard 9431, Soehnle Professional GmbH & Co. KG, Backnang, Germany) before and after each application.

Forty-eight hours after the last test AP application, volunteers visited DAaCRO's study facilities again to take a psychosocial stress test (TSST).

TSST

The TSST has been previously described [22, 23]. Briefly, the volunteer was asked to imagine that he or she has applied for a job and is now invited for an interview. The subject was advised to stand behind a microphone in front of a committee and give an impromptu speech with a preparation time of 3 min. To increase the social-evaluative threat of the situation, the participant was informed that he or she is video- and voice-recorded during the interview and that the two committee members are trained in behavioural observation. After the job interview, the volunteer was asked to assess present feelings of stress, anxiety and incertitude on a visual analogue scale (VAS). Next, the subject had to solve a mental arithmetic task during which he or she had to count backwards from 2023 in 17-steps as quickly and correctly as possible. In case of miscalculation, the volunteer had to start all over again. The TSST lasted 15 min.

Determination of sweat quantity

Volunteers were given functional shirts, which they wore during their time at the DAaCRO study facilities. Regarding TSST, perspiration was collected at two points in time using pre-weighed sweat-absorbent cotton pads (Cosmea, Pelz, Wahlstedt, Germany), which were placed under volunteers' armpits. Pads were folded and then secured under the left and right armpit of each study participant. One hundred and sixty minutes prior to the stress test, perspiration quantity was determined for 15 min during a resting state (baseline measurement). Afterwards, sweat pads were immediately enclosed in glass bottles and weighed using a high-precision balance (Soehnle professional standard 943). For later analysis, glass bottles were sealed using parafilm® (Brand GmbH & Co KG, Wertheim, Germany) and stored at −20°C. To investigate stress-induced sweat secretion, a fresh set of sweat-absorbing pads was worn during the 15 min of the TSST. After completion of TSST, pads were collected, weighed and frozen as described above.

Quantitative sniff test

The frozen sweat-absorbing pads were sent to the Beiersdorf Research Center (Hamburg, Germany) for evaluation of axillary malodour. Pads were thawed to room temperature, and malodour intensity was assessed by a panel of six trained sniffers using a 6-point intensity scale ranging from 0 (no sweat malodour) to 5 (extremely strong axillary malodour). Sniffers evaluated the intensity of axillary malodour from pads of the right and left armpit in direct comparison. Sniffers are trained twice a year [24].

Heart rate

The volunteers' heart rate was recorded continuously throughout TSST. Heart rate assessment started 20 min prior to the TSST, continued throughout the TSST, and ended 20 min after termination of the TSST. Assessment was carried out using a Polar watch device (S610i, S710i and S810i, Polar Electro GmbH, Büttelborn, Germany). The Polar watch recorded data every 5 s.

Determination of psychological parameters

Shortly before, during the TSST and immediately after the TSST, participants assessed their present feelings of stress perception, anxiety, insecurity, perspiration and body malodour by means of established questionnaires [25] on a 10-cm visual analogue scale (VAS) ranging from 'not at all' to 'highly'.

Collection and analysis of saliva cortisol samples

Volunteers collected saliva samples 2 min prior to the TSST as well as 1, 10, 20, 30, 45 and 60 min after the TSST using Salivettes® (Sarstedt, Nümbrecht, Germany). For analysis, frozen samples were thawed and centrifuged at 2000 *g* for 10 min. Free cortisol was assessed using a time-delayed fluorescence immunoassay in repeat determination [26].

Ergometer training (male volunteers receiving the gender-specific aerosol only)

After completion of the conditioning phase and prior to the AP application, male volunteers receiving the male-specific test AP aerosol visited DAaCRO GmbH & Co KG for assessment of general perspiration characteristics under physical activity. Volunteers were given functional shirts to wear and were asked to train on an ergometer (Kettler Ergometer PX1) for the duration of 25 min. According to the guidelines of the German Society for Sports Medicine and Prevention, ergometer tests were performed at temperatures between 18–24°C and at a relative humidity of 30–60% [27]. Exercise intensity was set at 60–70% of the maximum heart rate (HRMAX). To determine the individual HRMAX, the formula HRMAX = 220–age was used [28]. This method is one of the most commonly utilized methods worldwide to investigate changes of physiological parameters under physical activity.

After completion of the warm-up period of 10-min cycling, preweighed sweat-absorbent cotton pads were folded and then secured under the left and right armpit of each study participant as described above. Pads were worn for the rest of the ergometer test (15 min), immediately collected and stored in bottles. Bottles were sealed with parafilm® and frozen at −20°C for later analysis.

Olfactive stress sweat discrimination test (male volunteers receiving the gender-specific aerosol only)

This test was carried out to determine whether stress-induced malodour can be discriminated from malodour induced by physical activity. For that purpose, 2 sweat samples were obtained from each of the 19 male volunteers of the test AP aerosol group. Every sample pair was assessed by 20 untrained sniffers (10 female, 10 male). Sweat pads were collected after ergometer training (activity-induced sweating) and after the TSST (stress-induced-sweating). Sniffers were asked to intra-individually compare the pairs of malodours from the emotional vs. the activity states. Their task was to assign the correct sample to the stress-induced sweating. A further option was to decide that both samples smelled the same and a distinction was not possible.

Study II: Thermally induced sweating

This study was performed at the Beiersdorf Test Center (Beiersdorf, Hamburg, Germany) to determine the efficacy of the test AP roll-on

(see above) against thermally induced sweating. The study was carried out in accordance with the Declaration of Helsinki and ICH/GCP, and the guidelines for effectiveness testing of OTC antiperspirant drug products issued by the FDA [29] were followed. The study was conducted based on a randomized design and performed blind with respect to the test samples and open with regard to the untreated control area. The study was carried out under dermatological supervision. Eighty-two volunteers of both sexes were initially included in the study and the conditioning phase.

After completion of the wash-out period and prior to the first test AP application, baseline measurements were performed for assessment of axillary sweating. Volunteers were placed in a conditioned hot room for acclimatization (38.0 ± 1.0°C; 38 ± 5% relative humidity) for 40 min. At the end of this warm-up period, preweighed absorbent pads were placed in both axillae of each volunteer, sweat was collected for the next 20 min and pads were immediately weighed as described above. After the collection period, volunteers left the hot room and spent the next 60 min in an air-conditioned room under standard atmospheric conditions (21.5 ± 1.0°C; 45 ± 5% relative humidity).

Volunteers then visited the Test Center on four consecutive days for repeated standardized application of the test AP (see above).

Forty-eight hours after the final application of the test AP, volunteers again visited the Test Center and sweating was induced by thermal stimulation in the hot room for 40 min. After the warm-up period, perspiration was collected for 20 min. Pads were then removed and weighed. A total of 76 volunteers (40 women/36 men; age: 19–65 years) successfully finalized the study.

Statistical analysis

A significance level of 0.05 (alpha) was chosen for statistical analysis, based on two-sided hypothesis testing. For analysis, Microsoft Excel (XP; Microsoft Corp, Redmond, WA, U.S.A.), SAS software package for Windows V9.2 (SAS Institute Inc., Cary, NC, U.S.A.) and SPSS 17.0 (Systat Software Inc., San Jose, CA, U.S.A.) were used.

Sweat quantity and malodour were analysed with nonparametric tests: The Wilcoxon signed-rank test was used for paired samples and the Mann–Whitney *U*-test for independent samples. Individual sweat reduction for TSST as well as for hot room data was calculated as follows:

$$100\% \times [(m_{pad}(\text{untreated}) - m_{pad}(\text{treated}))/m_{pad}(\text{untreated})].$$

For normalization, the standard FDA hot room protocol takes the stimulated ipsilateral sweat amounts into account that are collected prior to product application during an additional hot room stay. However, such data cannot be obtained for the TSST due to possible stress adaptation. Out of this reason, the formula shown above was used to make these data comparable for both study types.

The mean sweat reduction per test group was calculated as the arithmetic mean of the individual sweat reduction values. Data are presented graphically with vertical box plots showing 10% percentile, lower quartile, median, upper quartile and 90% percentile. Outliers are presented as dots.

Participants – exclusions and outliers

Study I: Due to protocol deviations and outliers, the following volunteers were excluded from analysis: out of private reasons, one male assigned to the roll-on group and one male and one female allocated to the aerosol groups withdrew from the study even before product application.

Test AP roll-on: In one case (male), more than 150% of AP was applied at one visit. This high amount of applied AP may

Table I Physiological stress markers of roll-on, stick and aerosol AP groups. Heart rate data [bpm] are shown for measurements in upright position before the TSST and during the interview phase. Cortisol levels [nmol L^{-1}] were determined at 2 min before and 10 min after the TSST

		Roll-on			Stick			Aerosol		
Heart rate		**Prior**	**During**	**% Incr.**	**Prior**	**During**	**% Incr.**	**Prior**	**During**	**% Incr.**
Male	Mean	80.9	100.1	23.7	76.6	94.8	23.8	82.5	98.4	19.3
	SD	12.9	19.0		10.0	15.0		11.8	16.7	
	P			0.004			<0.001			0.002
Female	Mean	86.4	109.4	26.6	86.7	113.2	30.5	84.7	105.3	24.3
	SD	7.6	18.3		15.6	23.2		8.8	10.9	
	P			<0.001			0.001			<0.001
		Roll-on			**Stick**			**Aerosol**		
Cortisol		**Prior**	**After**	**% Incr.**	**Prior**	**After**	**% Incr.**	**Prior**	**After**	**% Incr.**
Male	Mean	3.20	12.53	292	3.17	10.17	221	5.43	18.40	239
	SD	1.86	8.07		1.71	4.70		4.22	8.75	
	P			<0.001			<0.001			<0.001
Female	Mean	3.82	9.79	156	3.82	9.91	159	4.40	7.43	69
	SD	2.70	5.33		2.45	7.06		2.61	4.78	
	P			<0.001			0.004			0.040

potentially influence perspiration and malodour. Out of that reason, data of this individual were excluded from AP efficacy calculations.

Test AP stick: In one case (male), more than 150% of AP was applied at one visit. Data of this individual were excluded from AP efficacy calculations. In one case (male), both sweat pads slid out of the axilla. As the temporary loss of sweat pads potentially influences the primary endpoints, data of this individual were excluded from analysis.

Test AP aerosol (female): In two cases, more than 150% of AP was applied at one visit. Data of these individuals were excluded from AP efficacy calculations.

Test AP aerosol (male): Two volunteers were excluded from analysis as their sweat difference between left and right axilla during TSST was >3 SD above the mean perspiration quantity. Outlier correction: one male was excluded because of extremely high absolute sweat amounts (>3 SD above mean perspiration quantity).

Results

Proof of physiological stress induction

Stress levels were assessed via determination of heart rate and saliva cortisol concentrations at different points in time before, during and after the TSST for all three test APs. In all groups, the TSST induced a substantial increase in heart rate and cortisol saliva levels in males and females (Table I). With respect to heart rate, this increase was comparable for both genders. Determination of cortisol concentrations displayed a higher stress-induced increase in males compared with females. Results of psychological questionnaires showed for both genders subjective self-perceived stress (data not shown).

Inhibition of sweat amount by test AP-products during the TSST

Analysis of endocrine and autonomous stress markers revealed that volunteers taking part in the TSST showed an observable stress reaction. This physiological reaction was paralleled by an increase in perspiration. Volunteers produced approximately 0.3 g of sweat per untreated axilla during the TSST indicating a strong increase in perspiration within a short period of time (15 min, amount of sweat/g: mean ± SD: male = 0.358 ± 0.236, female = 0.315 ± 0.225, all = 0.337 ± 0.230). Men displayed slightly higher values. However, this difference was not statistically significant (Fig. 1). During the TSST, all three test APs significantly reduced perspiration in the product-treated axilla independent of gender (% sweat reduction mean ± SD: male: roll-on: 51 ± 32, $P = 0.001$, stick 58 ± 40, $P = 0.005$, aerosol 33 ± 24, $P = 0.004$; female: roll-on 55 ± 29, $P = 0.001$, stick: 50 ± 26, $P = 0.001$, aerosol: 50 ± 22, $P = 0.002$). Within the same treatment group, differences between genders were not significant (Fig. 2).

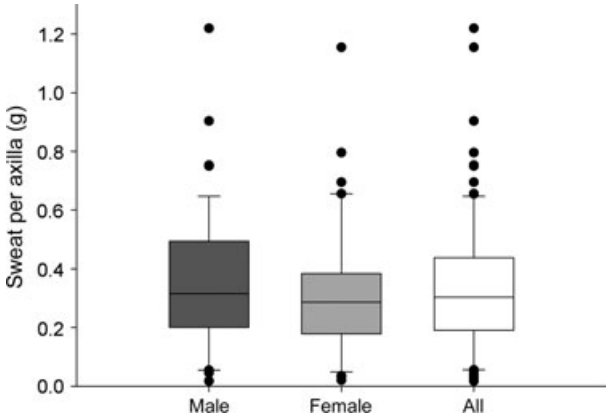

Figure 1 Sweat amount per untreated axilla during the TSST stress phase (female: $n = 44$, male: $n = 44$). Boxes indicate the 25th and 75th percentile; horizontal lines mark the median; whiskers represent the 10th and 90th percentile, and dots identify outliers.

Inhibition of sweat malodour by test AP formulations during TSST

For the investigation of effects of AP treatments on axillary malodour, trained sniffers assessed sweat pads obtained after the TSST from test AP-treated and untreated axillae of all three groups. After the TSST, sniffers rated malodour collected from untreated axillae as significantly more malodourous than malodour from AP-treated axillae. Compared with the control axilla, axillary malodour grading for the AP-treated armpits was significantly lower in males as well as females for all groups but one (male: roll-on: $P = 0.002$, stick $P = 0.013$, aerosol $P = 0.004$; female: roll-on $P = 0.004$, stick: $P = 0.598$, aerosol: $P = 0.003$; Fig. 3). Surprisingly, compared with the untreated axillae of the female test roll-on and aerosol groups, the untreated axillae of the female stick group showed a much lower score. Out of this reason, the corresponding malodour score for stick-treated axillae did not exhibit a significant difference.

Hot room data

To assess the efficacy of the test AP roll-on against thermally induced sweating, a hot room study was performed. As Fig. 4 illustrates, approximately 1.0 g sweat was produced in the control axillae during the stay in the hot room. Men displayed slightly higher values. However, this difference was not statistically significant. Figure 5 shows that the test AP roll-on significantly decreased the thermally induced sweat in the AP-treated axilla independent of gender (% sweat reduction mean ± SD: male = 26 ± 41, $P < 0.001$; female = 29 ± 54, $P < 0.001$; all = 28 ± 48, $P < 0.001$).

Olfactive stress sweat discrimination test

To determine whether emotionally induced sweat malodour (TSST) can be discriminated from thermally induced sweat malodour (ergometer training), 20 untrained volunteers assessed sweat samples obtained from the male test AP aerosol group. A pairwise comparison of thermal and stress sweat samples each from the same donor was carried out. Results showed that sweat amounts produced by physical activity exceeded the amount of sweat generated during the TSST. The mean sweat amount released from the control axillae during ergometer cycling was 0.601 ± 0.587 g. During the TSST, a mean sweat amount of 0.402 ± 0.309 g was collected. By intra-individual comparison of the sweat samples, the untrained volunteers were able to identify stress sweat with a higher than by-chance probability (mean value: 56.32 ± 13.35; $P = 0.0478$).

Also, the trained sniffer panel rated the pads obtained after the TSST significantly higher in malodour intensity than the ergometer

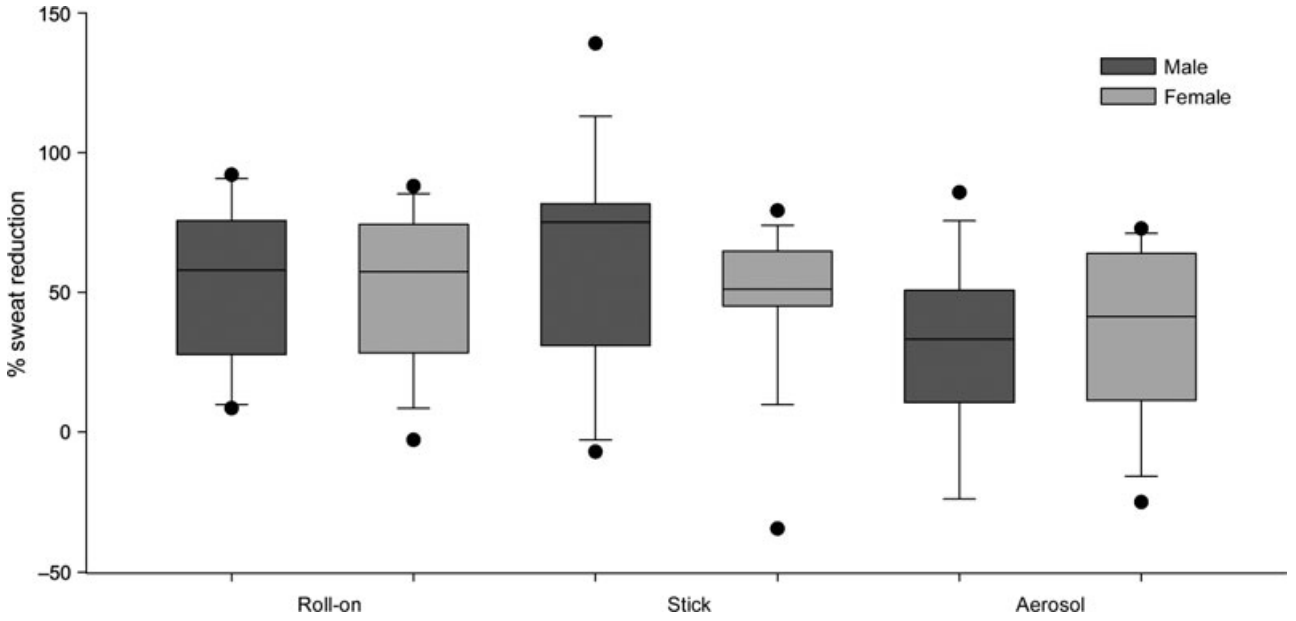

Figure 2 Sweat reduction by the test AP roll-on, stick and aerosol in the treated axilla during the TSST. Data were normalized to the sweat amount in the untreated axilla (male: roll-on: $n = 13$, stick: $n = 13$, aerosol: $n = 16$; female: roll-on: $n = 15$, stick: $n = 15$, aerosol: $n = 12$). Boxes indicate the 25th and 75th percentile; horizontal lines mark the median; whiskers represent the 10th and 90th percentile, and dots identify outliers.

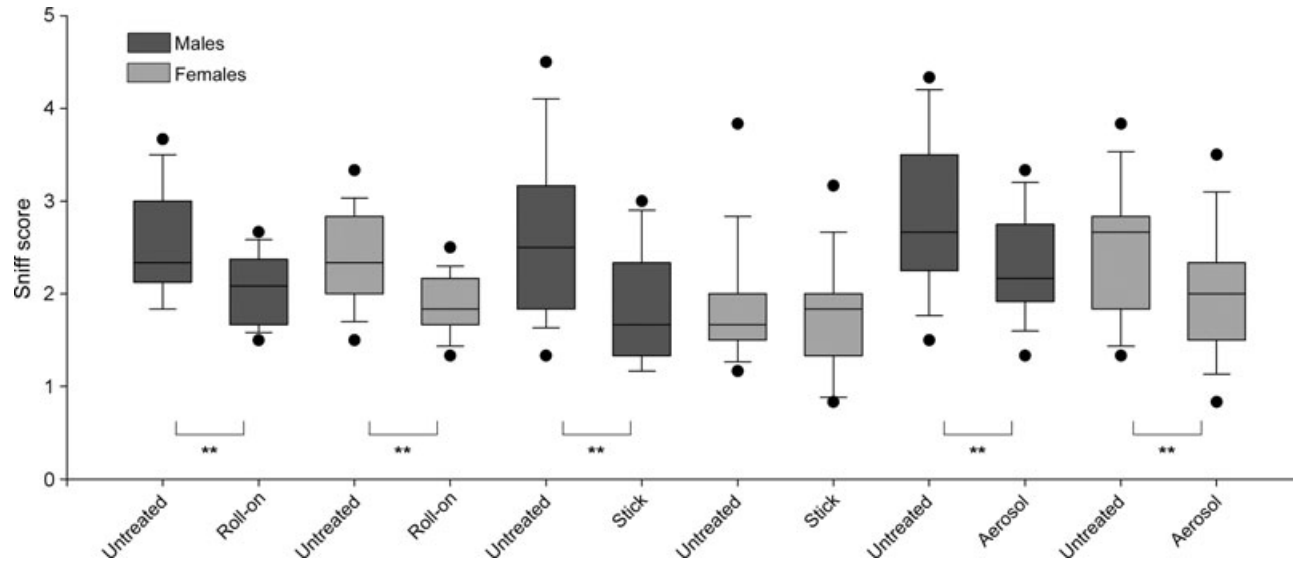

Figure 3 Malodour grading after TSST performed by a trained sniffer panel for test AP roll-on, stick and aerosol. Data for male and female volunteers shown separately. Boxes indicate the 25th and 75th percentile; horizontal lines mark the median; whiskers represent the 10th and 90th percentile, and dots identify outliers. Asterisks indicate significant differences (**$P < 0.01$).

pads (TSST: mean sniff score: 2.77 ± 0.88; ergometer cycling: mean sniff score: 2.13 ± 0.58; $P = 0.002$).

Discussion

Stress sweating in the underarm region is triggered in emotionally challenging situations. Out of this reason, it is experienced by all individuals and can occur in everyday situations independently of thermally induced perspiration. To combat undesired stress sweat production and the resulting characteristic unpleasant sweat scent, specially developed Deo/AP formulations are needed. Various AP product forms including roll-ons, sticks, aerosols, gels, soft solids, pump sprays, powders and creams are marketed. According to Euromonitor data, in 2011, the highest market shares worldwide

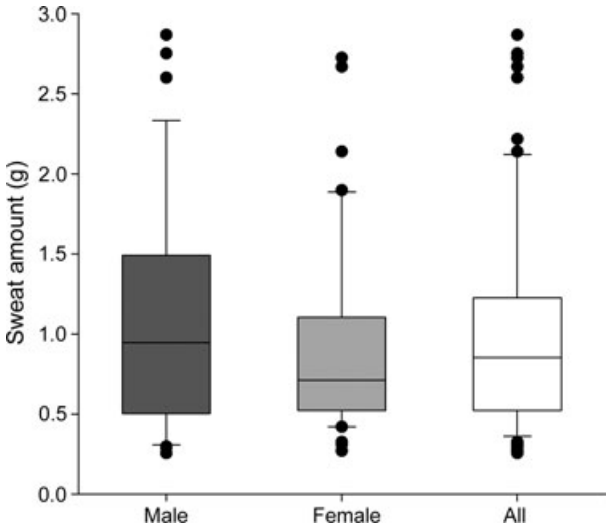

Figure 4 Sweat production during 40-min hot room (male: $n = 36$, female: $n = 40$, all: $n = 76$). Boxes indicate the 25th and 75th percentile; horizontal lines mark the median; whiskers represent the 10th and 90th percentile, and dots identify outliers.

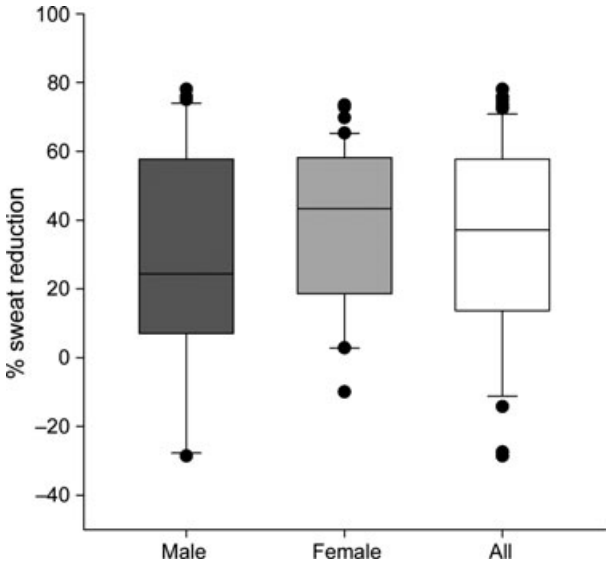

Figure 5 Sweat reduction in the roll-on AP-treated axilla during the stay in the hot room normalized to the sweat amount released from the untreated contralateral axilla. Boxes indicate the 25th and 75th percentile; horizontal lines mark the median; whiskers represent the 10th and 90th percentile, and dots identify outliers.

were attributed to aerosols (43.4%), roll-ons (26.5%) and sticks (18.1%) [30]. Using habits of APs vary dependent on individual and regional preferences.

Not all product forms exhibit the same degree of efficacy when comparing equal concentrations and amounts of aluminium salts. Aqueous and hydroalcoholic solutions, for example, have been shown to be more efficacious than water-free formulas [31]. Emulsions range in between these two alternative approaches with o/w emulsions being superior to w/o emulsions. This ranking is due to the fact that the aluminium ions have to be dissolved in an aqueous phase to be able to diffuse into the sweat duct. In case an AP formula does not contain water, the formulation can take in humidity derived from the sweaty skin. This process then leads to the dissolution of the aluminium salts [31, 32].

Stress-induced sweating and thermal sweating exhibit characteristic differences [33]. Stress-activated sympathetic neurons and possibly circulating adrenal catecholamines rapidly stimulate sweat glands enabling the body to react to threatening or unpleasant situations within seconds to minutes. Out of this reason, sweat amounts secreted within the first minutes under stress can be considerably higher than sweat amounts produced in response to a thermal challenge, which can be experienced in everyday situations. In a warm environment, perspiration rates rise only slowly with increasing body core temperature in particular in non-heat-adapted individuals [34, 35].

Moreover and in contrast to purely thermal sweating, upon activation, apocrine glands release substances that generate malodour after bacterial metabolization. The distribution and vaporization of already existing and newly formed volatile malodour molecules are further promoted by eccrine aqueous sweat.

To address the challenges of stress sweating described above, we developed specifically designed formulas and incorporated, for example, highly effective ACH or aluminium zirconium glycinate (AZG) in combination with ASCH as AP active ingredients as well as an additional zinc compound to oppose bacterial growth and subsequent malodour formation.

Their efficacy against stress-induced sweating and malodour production was determined after 48 h, which has become the market standard for efficacy claims. As personal preference regarding the delivery system plays a crucial role in the choice of an AP, we designed formulas for the three most popular application forms – roll-on, stick and aerosol – and tested them unperfumed.

We provoked stress-induced sweating and malodour generation using the widely accepted TSST [22, 23, 36]. To verify that the psychosocial stress during TSST was perceived by all volunteers, physiological stress reactions such as heart rate and saliva cortisol concentrations were determined at different points in time before, during and after the TSST. Increasing heart rates and an augmentation of saliva cortisol levels clearly indicated a substantial stress reaction. Regarding heart rates, this increase was comparable for both genders. With respect to induced cortisol levels, however, men showed a greater increase than women [37]. In the literature, some authors report similar findings of elevated male cortisol concentrations [38, 39] whereas others did not observe any gender differences [40]. Additionally, results of psychological questionnaires documented that both genders experienced subjective self-perceived stress during the TSST.

The elevation of endocrine and autonomous stress markers during the TSST was paralleled by a substantial sweat production in the untreated axillae of both males and females. Our results demonstrate that all three test APs significantly decreased the amount of sweat in the treated axillae independent of gender. This is of special significance as we show here for the first time an effective reduction in stress-induced perspiration 48 h after product application. The test AP aerosol performed less well compared with test AP roll-on and stick. As the tested AP aerosols were formulated as water-free suspensions, this observation is in line with the fact that

the efficacy of an aluminium salt is higher in water-containing systems compared with anhydrous formulations [31]. In contrast, application of the AP stick, also a water-free formula, achieved the highest percentage in sweat reduction in this study, at least for the male study group. However, a direct comparison of different formulas is only valid if the same type and amount of active are deposited on the axillary skin, which is in this study not the case for stick and aerosol APs.

With respect to AP effects on malodour production, trained sniffers assessed malodour collected after the TSST from the untreated axillae as significantly more malodourous than malodour from the AP-treated axillae. Compared with the control axilla, the AP-treated armpits showed significantly lower scores for males as well as females for all three AP groups but one. In the study group of females treated with the AP stick, the mean sniff score value obtained for the untreated axilla after stress (1.86) was rather low compared with untreated axillae in the female roll-on (2.41) and aerosol groups (2.67). Because the assignment of volunteers to a test group was carried out at random and there is no evidence for contralateral effects of topical APs in the literature, the low sniff score for the control area in the female stick group is judged as incidental. The mean sniff score of the stick-treated female axillae (1.74) on the other hand exhibited a level comparable to the female roll-on (1.88) and aerosol (2.13). However, comparing untreated and stick-treated axillae, no statistical significance could be achieved.

As shown in Figs 2 and 3, substantial interindividual differences in sweat and malodour production were observed. Large individual differences in sweat secretion and/or malodour generation lead to a variability of data. When interpreting results, one also has to keep in mind that the amount of sweat was determined gravimetrically using cotton pads. This procedure is currently regarded as state of the art. However, artefacts may occur in some cases. For instance, the fixation of pads in the armpit can be altered. This situation can appear in particular when volunteers press their arms against the upper part of the body influencing sweat flow by exerting pressure on the underlying soft tissue [20]. Also, pads may slip and slide out of place. In this case, less sweat is absorbed by the pad. Out of this reason, even negative values for sweat reduction can be obtained after normalization to controls. To make these phenomena transparent, we depicted all outliers in the respective figures.

To be able to judge the sweat reducing efficacy of the formulas achieved during the TSST, we exemplarily performed an additional assessment of the roll-on in the hot room. The roll-on was selected as a reference because its aqueous formula regularly scores on a very high level in hot room studies (internal unpublished data). This study was performed following the FDA guidelines for effectiveness testing of OTC antiperspirant drug products [29] mentioned above with an interval of 48 h between last product application and thermal challenge analogous to the TSST study. During the stay in the hot room, both male and female volunteers produced a substantial amount of thermally induced sweat. After 48 h, independent of gender, the test AP roll-on significantly decreased sweat in the AP-treated axilla as compared to the control axilla (mean reduction 28%). When comparing the data from stress-induced to thermally induced sweating, it becomes obvious that the stress sweat reduction brought about by all three AP formulations was even more pronounced than the decrease in thermally induced sweating achieved by the roll-on.

As we here present data 48 h after product application, it is difficult to compare our results with previous data from the literature. So far, most published data have only been obtained for a 24h application interval. Moreover, the amounts of applied products are rarely identical.

Without specifying the products and amounts used, Bowman and co-workers, for example, showed in six independent hot room studies, 24 h after the last of four AP applications, sweat reduction rates of 20–42% [41]. Recently, for a commercial 'clinical strength' soft solid, 48% sweat reduction was demonstrated after 24 h (4 × 600 mg per axilla) [42]. Also, 4 h after the application of an aerosol (4 × 1200 mg incl. propellant), teenagers showed a sweat reduction of 31% during the TSST [22].

The special function of odouriferous axillary stress-induced secretions as an evolutionarily conserved means for non-verbal communication has recently been reviewed [33, 43]. Having paired samples of activity- and stress-induced sweat, each obtained from the same individuals, at hand, we wondered whether TSST pads and pads obtained during ergometer training of the male test AP aerosol group could be distinguished by untrained volunteers of both genders. In fact, volunteers were able to identify stress sweat samples with a higher than by-chance probability. These findings are in line with data showing that subjects were, for example, able to discriminate between pads worn during a frightening opposed to a non-fearful movie [44, 45]. Following a stressful real-life situation such as the TSST or axillary epinephrine injections, thiol-containing molecules were found to be concentrated in the respective sweat samples [46]. The way sweat scent of different origin is perceived by other individuals, and the fact that stress sweat is experienced as more malodourous than thermally induced sweat makes the development of a reliable stress sweat protective AP all the more important.

In conclusion, we were able to show, for the first time, a highly effective reduction in emotionally induced axillary sweating for three different topical AP formulas 48 h after product application. The roll-on, the stick and the aerosol substantially reduced both underarm wetness and malodour. Also, as shown exemplarily for the roll-on, thermally induced perspiration was effectively decreased.

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