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Article

Significant Reduction of Body Odor in Older People with a pH 4.0 Emulsion

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Abstract: The impact of increasing age on body odor has become an important issue as our understanding of underlying skin changes in older people has increased. Therefore, cosmetic skin products especially for the needs of the elderly are of growing importance. This randomized single-blind crossover study assessed the deodorizing efficacy of two cosmetic products with different pH values on the age-specific odor of an elderly female subject panel (≥60 years). The two test products, adjusted to pH 4.0 and pH 5.8 were applied to the axillae once daily for three consecutive days after standardized washing of the axillae. The untreated axilla was used as a control. Six odor judges evaluated the efficacy of both products. Additionally, bactericidal and fungicidal activity was investigated with *in vitro* microbiologic tests. The pH 4.0 water in oil (W/O) emulsion significantly reduced axillary malodor in 44 elderly subjects at 8 and 24 h after treatment, compared with controls (untreated axillae) ($p < 0.001$ after 8 and 24 h), whereas pH 5.8 emulsion had no effect ($p = 0.441$ after 8 h; $p = 0.425$ after 24 h). Moreover, the pH 4.0 emulsion reduced axillary malodor at 8 and 24 h after treatment, compared with the pH 5.8 emulsion just narrowly missing statistical significance ($p = 0.078$ after 8 h; $p = 0.053$ after 24 h). Microbiologic *in vitro* tests showed that the pH 4.0 emulsion reduced the levels of odor-producing bacteria *S. epidermidis* and *C. minutissimum* after 1 h (2.98 log and 4.25 log). After 24 h, levels of *S. aureus* (>5.50 log), *P. acnes* (>5.30 log) and

E. coli (>5.46 log) were further reduced whereas no effect was observed for pH 5.8. A pH 4.0 emulsion significantly reduced axillary malodor for up to 24 h after application in females aged at least 60 years. This reduction in malodor is very likely due to a reduction of odor-producing bacteria.

Keywords: axillary malodor; skin pH; pH 4.0 emulsion; aging skin

1. Introduction

Human body malodor and products to prevent malodor have long been topics of studies. Body odor is caused by degraded skin components accumulating on the skin surface as well as secretions from the sweat and sebaceous glands and is affected by the skin pH [1,2]. Skin pH is an important factor for skin integrity and wound healing. It ranges from as low as 4.0 up to 6.3, as reviewed by Lambers *et al.*, which is in contrast to the approximately neutral pH of the body's internal environment [3–5]. The skin pH is influenced by several endogenous factors, such as skin moisture, sweat, sebum, anatomic site, genetic predisposition, race, age, atopic skin diseases, irritant dermatitis, diaper dermatitis, diabetes, and uremia [6]. Furthermore, there are also various exogenous factors—soaps, detergents, cosmetic products, occlusive dressings, skin irritants and topical antibiotics—which influence the pH of the skin and therefore possibly impact body odor [6].

The effect of increasing age on malodor has been a controversial subject in the past but recently this issue has gained attention due to an increased understanding of underlying skin changes. Schreml *et al.* demonstrated that aging causes an increase in skin pH which affects the skin microbiome and may thus change body odor [7]. Therefore, the development of cosmetic skin products especially for the needs of older people is of growing importance.

So far, only a few studies have investigated the effects of cosmetic skin products on body odor and even fewer have focused on malodor and aging skin. Stenzaly-Achtert *et al.* showed that there are pH differences in different body regions and revealed the correlation between a high skin pH and the growth of several microorganisms that produce malodor [8].

This study aimed to demonstrate that an acidic pH of skin care products is crucial in particular with respect to body odor or malodor, respectively. We hypothesized that an acidic water in oil (W/O) emulsion, which has proven properties in decreasing skin pH [9], may also change the skin microbiome and thus impacts positively body odor in elderly.

2. Results

2.1. Demographic Data

Subjects included in the study had a mean age of 67.3 years (standard deviation, SD = 3.9 years) and were exclusively female. The following analysis sets were defined: Screening Population (n = 46), Safety Population (SP), and Per Protocol (PP) population. The SP (n = 45) included all subjects who were enrolled into the study and who received at least the first dose of study treatment. The PP population (n = 44) included all randomized subjects who finished the study without major protocol

violations such as disrespect of inclusion/exclusion criteria, use of a therapy which could compromise study results, or poor compliance in study treatment administration or protocol requirements.

2.2. Efficacy Analysis

The efficacy endpoint of axillary odor, as assessed on a visual analog scale (VAS), was evaluated in the PP population alone. Table 1 presents the mean malodor VAS scores of axillary sweat and the corresponding changes from baseline. Changes from baseline at 8 and 24 h are also shown as a bar graph (Figure 1). One subject was accidently treated with the test product in each axilla instead of leaving one axilla untreated as a control in the first crossover period (period I). Thus, all data for this subject during period I were excluded from analysis. Another subject did not go to the scheduled follow-up appointment on day 10 of the study due to personal reasons. As this protocol deviation was classified as a major deviation, no data were used from this subject for analysis.

Table 1. Axillary malodor scores (mm). Mean scores and changes from baseline, as assessed by six odor judges (*n* = number of subjects).

Period	Treatment	*n*	Mean Scores			Mean Changes from Baseline	
			Baseline (24 h)	After 8 h	After 24 h	After 8 h	After 24 h
I	Untreated area	43	26.8 ± 10.1	21.1 ± 7.3	25.8 ± 6.9	−5.7 ± 7.9	−0.9 ± 7.5
	pH 4 emulsion	21	27.5 ± 9.3	17.6 ± 3.9	23.3 ± 4.7	−9.9 ± 8.6	−4.2 ± 7.8
	pH 5.8 emulsion	22	24.5 ± 7.1	18.8 ± 6.1	23.3 ± 6.9	−5.7 ± 5.9	−1.2 ± 7.4
II	Untreated	44	21.2 ± 5.3	19.6 ± 6.0	22.3 ± 7.5	−1.6 ±4.6	1.1 ± 5.6
	pH 4 emulsion	22	21.6 ± 7.7	17.2 ± 3.8	18.1 ± 4.2	−4.4 ± 7.5	−3.6 ± 6.5
	pH 5.8 emulsion	22	23.4 ± 9.6	19.8 ± 4.3	22.5 ± 5.6	−3.6 ± 9.5	−0.9 ± 6.8
Both periods	Untreated area	44	23.9 ± 6.9	20.3 ± 6.1	24.1 ± 6.1	−3.6 ± 4.9	0.2 ± 4.4
	pH 4 emulsion	43	24.5 ± 8.9	17.4 ± 3.8	20.6 ± 5.1	−7.1 ± 8.5	−3.9 ± 7.1
	pH 5.8 emulsion	44	23.9 ± 8.4	19.3 ±5.2	22.9 ± 6.2	−4.7 ± 7.9	−1.0 ± 7.0

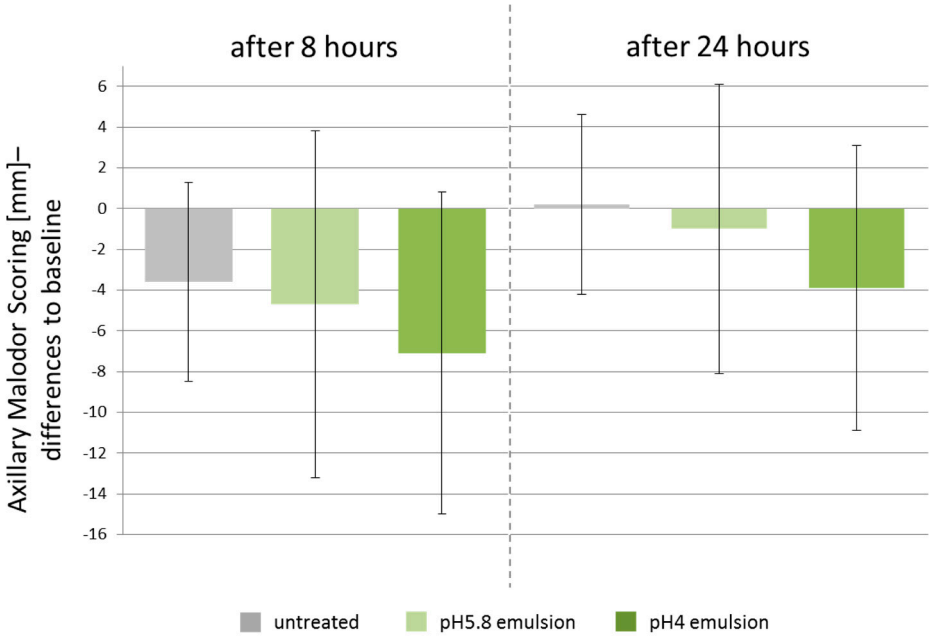

Figure 1. Overall changes from baseline in axillary malodor scores at 8 and 24 h.

In the repeated ANOVA measurements of baseline values, significant differences only were found between odor judges ($p < 0.001$), between periods ($p < 0.001$), and for the interaction between odor judges and periods ($p < 0.001$). For the assessment at 8 h after the last product application, significant differences were found between treatments ($p = 0.001$), odor judges ($p < 0.001$), and for interactions between odor judges and periods ($p < 0.001$), between odor judges and axillary sides ($p < 0.001$), and between periods and axillary sides ($p = 0.019$). For the assessment at 24 h after product application, significant differences were found between treatments ($p < 0.001$), odor judges ($p < 0.001$), periods ($p < 0.001$), and for interactions between treatments and odor judges ($p = 0.016$), between treatments and axillary sides ($p = 0.033$), and between odor judges and periods ($p < 0.019$). As the interaction between treatments and periods was not significant at any time point, no "memory" effect regarding behavior was detected. Therefore statistical analysis of this crossover trial was performed using data including all periods. Table 2 presents the results of treatment comparisons when analyzed with the Tukey's test, and the results of comparisons of post-treatment assessment times *versus* baseline when analyzed with paired *t*-tests. The pH 4.0 emulsion resulted in a significantly lower axillary malodor score at 8 and 24 h after the last of three treatments when compared with the untreated axilla. Compared with the pH 5.8 emulsion, the pH 4.0 emulsion resulted in numerically lower mean axillary malodor score at 8 and 24 h after the last application. However, these differences were not significant when using a significance level of 0.05. When comparing assessment times *versus* baseline with paired *t*-tests, both emulsions had significantly lower axillary malodor scores at 8 h, compared with the untreated axilla. However, after 24 h, axillary malodor was only significantly reduced compared to baseline after treatment with the pH 4.0 emulsion.

Table 2. Statistical results of axillary malodor scoring. Results of Tukey's test when comparing treatments, and paired *t*-test when comparing time points. * significant, $p \leq 0.05$; $^{n.s}$ not significant; NA, not assessed.

Treatment	*p*-values of Tukey's Tests				*p*-values of Paired *t*-tests	
	After 8 h		After 24 h			
	pH 4.0 Emulsion	pH 5.8 Emulsion	Ph 4.0 Emulsion	pH 5.8 Emulsion	After 8 h *vs.* Baseline	After 24 h *vs.* Baseline
Untreated area	<0.001 *	0.441 $^{n.s}$	<0.001 *	0.425 $^{n.s}$	<0.001 *	0.815 $^{n.s}$
pH4.0 emulsion	NA	0.078 $^{n.s}$	NA	0.053 $^{n.s}$	<0.001 *	<0.001 *
pH5.8 emulsion	NA	NA	NA	NA	<0.001 *	0.329 $^{n.s}$

2.3. Safety Analysis

The safety analysis was based on the SP only. No serious adverse events (SAE) and no adverse events (AEs) were reported during the study period.

2.4. Microbiological Results

The *in vitro* analysis showed reductions in levels of *S. epidermidis* and *C. minutissimum* after one hour for the pH 4.0 emulsion, but not for the pH 5.8 emulsion. The bactericidal effect of the pH 4.0

emulsion compared to the pH 5.8 emulsion is shown in Table 3. After 24 h, levels of *S. aureus*, *P. acnes*, and *E. coli* were also significantly reduced by the pH 4.0 emulsion, but not by the pH 5.8 emulsion.

Table 3. Bactericidal effect of the pH 4.0 emulsion. Overview of the log-reductions of the quantitative suspension tests for bactericidal activity (EN 1040) and fungicidal activity (EN 1275). Results which passed the required criteria of >5.00 log response >4.00 log are given in bold numbers.

Time	**1 h**		**4 h**		**24 h**	
pH	**pH 5.8**	**pH 4.0**	**pH 5.8**	**pH 4.0**	**pH 5.8**	**pH 4.0**
S. epidermis	<1.10	2.98	<1.10	**4.59**	<1.10	**>5.48**
S. aureus	<1.13	<1.13	<1.13	<1.13	<1.13	**>5.50**
C. minutissimum	<1.01	**4.25**	<1.01	**>5.39**	<1.01	**>5.39**
M. furfur	<0.16	<0.16	<0.16	<0.16	<0.16	1.05
P. acnes	<0.93	<0.93	<0.93	**>5.30**	<0.93	**>5.30**
Candida albicans	0.10	0	0.16	0.77	0.18	1.69
T. rubrum	<0.04	0.37	<0.04	0.79	<0.04	1.41
E. coli	<1.09	1.81	<1.09	**>5.46**	<1.09	**>5.46**

3. Discussion

The present study revealed that a W/O emulsion with pH 4 is effective in reducing body odor significantly whereas the W/O emulsion with pH 5.8 was not effective compared to untreated control. Moreover, the application of the pH 4.0 emulsion resulted in numerically lower malodor scores at 8 and even after 24 h following the last treatment application, compared to the pH 5.8 emulsion. However, pH 5.8 emulsion had also significantly lower axillary malodor scores after 8 h, but this may be depend on the properties of the W/O emulsion alone. The differences in malodor scores between treatments just narrowly missed statistical significance.

Based on the current knowledge that skin acidification is important for epidermal barrier function [4,5], pH reduction in the elderly is reasonable, because skin pH rises with increasing age and impacts skin function negatively. Skin pH is also higher in intertriginous areas such as the axillae [10]. Moreover, reduction of skin pH has been shown to improve skin barrier, regenerate transepidermal water loss (TEWL), and positively impact the skin microbiome. As well as the skin pH, the composition of the human skin flora is also altered during the aging process [10]. Bacteria appear during aging and have been associated with malodor production [11] and an increased bacterial activity in the axillae of subjects was observed with a pungent axillary malodor [12]. *In vitro* and *in vivo* studies of axillary odor and skin flora of 34 subjects revealed aerobic bacteria within the axillae to be responsible for high malodor intensity [13]. The results of the present study are in line with this finding, as the pH 4.0 emulsion was able to reduce the activity of different bacterial strains putatively involved in malodor [2,14]. Consequently, the reduction in odor-producing bacteria appeared to lead to a concurrent reduction in malodor. But also the reduction of commensal bacteria like *S. epidermis* or *S. aureus* is of clinical relevance, considering their role in skin diseases [15]. Additionally, a more acidic resident skin microbiome is important as it provides protection from pathogens [16].

With regard to these last two aspects, a pH 4.0 emulsion seems to be a more effective cosmetic product compared to emulsions with higher pH values, and is also well tolerated.

4. Materials and Methods

4.1. Study Design

This study was a randomized single-blind exploratory crossover study which contained an additional *in vitro* substudy. Subjects were randomly assigned to receive one of the two test products in period I which was followed by the alternative treatment in period II. Axillae were also randomized to receive active treatment or remain untreated and serve as a negative control. Randomization was performed using randomly permuted blocks of fixed size. In the *in vitro* part of the study, microbiologic tests were performed to investigate the axillary bacterial growth.

4.2. Participants

Participants were female and at least 60 years of age. Drug addicts or alcoholics were not included in the study. Other exclusion criteria were AIDS or infectious hepatitis (if known to the subjects), cancer, active skin disease at test area, documented allergies to cosmetic products, systemic therapy with antibiotics within the last two weeks, heavy smoker, participation or being in a waiting period after participation in similar cosmetic and/or pharmaceutical studies. Subjects with asthma or hypertension were only included if they were medicated.

4.3. Restrictions

Throughout the course of the study, the subjects were not allowed to use detergents (except for the perfume-free soap provided by the test facility), deodorants or antiperspirants in the axillae. During the test phase, the participants were instructed to discontinue washing their axillae at home, as washing of this area was exclusively performed at the study site. Furthermore, they had to remove their axillary hairs once on study day 1. The participants were not allowed to smoke, to use hair spray or other perfumed substances on sweat odor assessment days. Additionally, the subjects were instructed not to eat spicy foods, including garlic and onions, not to drink alcohol, and not to wear clothes washed with perfumed washing agent or fabric softener during the entire test phase. Hard physical exercise with heavy sweating, sauna and swimming during the test phase were also to be avoided.

4.4. Concomitant Medication

Any treatment that was not listed in the exclusion criteria was allowed at the discretion of the investigator. Medications for regulation of thyroid function were allowed. All study-relevant prior and concomitant medication were documented. All diseases which occurred during the study period had to be treated according to standard medicinal practice. The study-relevant disease and the treatment were documented. If the treatment was not allowed during the study, the subject was excluded from further participation.

4.5. Test Materials and Application

The test products (pH 4.0 emulsion and pH 5.8 emulsion) are cosmetic products (Table 4). The test materials were applied in a volume of 800 μL to the axilla by a technician with a Finnpipette. The applied product was evenly distributed by the technician. The untreated area was used as a control.

Table 4. Constituents of the formulations used in the clinical study.

pH 4.0 Emulsion	pH 5.8 Emulsion
Sorbitan oleate	Sorbitan oleate
Polyglyceryl-3 Polyricinoleate Isohexadecane	Polyglyceryl-3 Polyricinoleate Isohexadecane
Ethylhexyl Stearate	Ethylhexyl Stearate
Decyl Oleate	Decyl Oleate
Sucrose Polystearate	Sucrose Polystearate
Glycerol 85%	Glycerol 85%
Magnesium sulfate	Magnesium sulfate
Water, purified	Water, purified
Ammonia 25%	–
Glycolic acid 70%	–

4.6. Test Procedure

The study started with a wash-out-phase on days 1–7 with perfume-free soaps. On day 8 the subjects returned to the study site and performed a protocol-defined wash of each axilla with perfume-free soap for approximately 10 s. Following the wash, they rinsed their axillae with water, and patted them dry with a fresh paper towel under the supervision of a technical assistant. On day 9 (Baseline Period I), 24 h after the protocol-defined wash, the baseline sweat odor was assessed by so-called "odor-judges" followed by another protocol-defined wash of their axillae and the first application of the study treatment according to a random assignment. On day 10, subjects performed another protocol-defined wash followed by another application of study treatment. This procedure was repeated on day 11. About 8 h after product application on day 11, assessment of sweat odor was performed by the odor judges to evaluate product efficacy. This was repeated on day 12, approximately 24 h after the last product application. Afterwards, subjects underwent the wash-out-phase at home for the following three days. On day 15, subjects repeated the procedures performed on day 8 and started the baseline period II on day 16. Days 16–19 were performed in a similar manner to days 9–12. In each application period, one axilla was treated and the other remained untreated.

4.7. Axillary Malodor Evaluation by Odor Judges

Six odor judges performed a comparative assessment of sweat odor directly in both axillae of the participating subjects using a VAS ranging from 0 to 100 mm, where 0 mm corresponds to "no sweat odor" and 100 mm corresponds to "most extreme sweat odor". The odor judges were considered to have a higher-than-normal olfactory ability to detect and quantify sweat odor. In order to qualify, they sniffed and rated five different concentrations of isovaleric acid, as the smell of this acid is similar to a

typical sweaty odor [12]. Only subjects with no failures in this ranking were able to take part in sniff tests as odor judges.

4.8. Safety Analysis

Analysis of safety and tolerability included study-relevant adverse events (AEs), deterioration of comorbidities, and reasons for termination.

4.9. Ethics Statements

In accordance with the Declaration of Helsinki, the subjects provided written informed consent to participate in the study. Before initiating the trial, the Investigator had written and dated full approval from the Freiburg Ethics Commission International for the protocol, protocol amendment(s), if applicable, and the subject informed consent form.

4.10. Statistical Analysis

Computation of the statistical data was carried out with SAS for Windows. A significance level of 0.05 (alpha) was chosen for statistical analysis. Due to the explorative character of this study, no further adjustment for multiplicity was done. Homogeneity of baseline values of the two application periods was inspected by running an ANOVA with blocked subject factors, the factors of odor judge (6 levels), axillary side (2 levels: right, left), period (2 levels: day 9, day 16) and their first levels of interactions. Statistical analyses for sniff values were performed for each post-treatment assessment time point with ANOVA and Tukey's test as post-tests. Paired *t*-tests were performed to compare each treatment to baseline on mean values over odor judges.

4.11. In Vitro *Study: Microbiological Tests*

The *in vitro* part of the present study were performed by Labor L&S AG (Bad Bocklet, Germany) investigating the association between bactericidal and fungicidal activity and the pH of the emulsion used. The quantitative suspension tests EN 1040 and EN 1275 (date of: March 2006) were used to evaluate bactericidal activity and fungicidal activity, respectively, through the dilution-neutralization method. Test strains for the bacteria *Staphylococcus (S.) epidermidis*, *S. aureus*, *Corynebacterium (C.) minutissimum*, *Propionibacterium (P.) acnes*, and were selected as resident flora of the axilla putatively responsible for body odor [2,14,17]. Test strains for *Escherichia (E.) coli*, and the yeasts *Candida albicans*, *Malassezia (M.) furfur*, and *Trichophyton (T.) rubrum* were selected according to their incidence on human skin [17]. Strains were evaluated after exposure times of 1 h, 4 h, and 24 h with the test product or the comparator product, both of which were at a concentration of 80% (=undiluted). Changes from baseline had to be higher than the required criteria of >5.00 log response >4.00 log in order to be considered significant.

5. Conclusions

Lowering of an increased skin pH to physiological values affects skin functions, e.g., improving the epidermal barrier. Moreover, the present study reveals further beneficial properties of a pH 4 W/O

emulsion. This newly developed pH 4 W/O emulsion exhibits antibacterial effects and significantly improves thereby associated body malodor. Therefore, skin care emulsions adjusted to an acidic pH of 4 very likely provide a new active principle for cosmetic products, in particular for the elderly.

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Author Contributions

The tested products were formulated according to the requirements by Ulrich Knie from Dr. August Wolff GmbH & Co. KG Arzneimittel. The study was designed by Michael Kemper and Christoph Abels from Dr. August Wolff GmbH & Co. KG Arzneimittel and performed by Stephan Bielfeldt and Klaus-Peter Wilhelm of proDERM. Microbiological testing was performed by Labor L&S AG. The authors reviewed and finalized the draft manuscript.

Conflict of Interests

Christoph Abels, Ulrich Knie and Michael Kemper are employees of August Wolff Arzneimittel GmbH & Co. KG (sponsor of the study). Stephan Bielfeldt and Klaus-Peter Wilhelm are employees of proDERM (investigator of the study).

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