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Impact of a Glycolic Acid-Containing pH 4 Water-in-Oil Emulsion on Skin pH

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Key Words
Skin pH · Diabetics · Glycolic acid emulsion

Abstract
The skin pH is crucial for physiological skin functions. A decline in stratum corneum acidity, as observed in aged or diseased skin, may negatively affect physiological skin functions. Therefore, glycolic acid-containing water-in-oil (W/O) emulsions adjusted to pH 4 were investigated regarding their effect on normal or increased skin pH. A pH 4 W/O emulsion was applied on three areas with pathologically increased skin surface pH in diabetics (n = 10). Further, a 28-day half-side trial (n = 30) was performed to test the long-term efficacy and safety of a pH 4 W/O emulsion (n = 30). In summary, the application of a pH 4 W/O emulsion reduced the skin pH in healthy, elderly and diabetic subjects, which may improve epidermal barrier functions.

Introduction

A proper regulation of skin pH is crucial for physiological skin functions such as integrity/cohesion of the stratum corneum (SC), homeostasis of the epidermal barrier and antimicrobial defense [1–4]. Aging is a major endogenous factor that leads to increased skin pH [5, 6]. A direct correlation between aging and the pH measured on skin surface (pH_{SS}) has been described [2, 7–9]. The altered pH_{SS} is supposed to be linked to clinical symptoms like rough and dry skin, which is sometimes associated with itching, as well as increased skin infections and susceptibility to contact allergies [10, 11].

A further endogenous factor with increasing prevalence is diabetes mellitus. Yosipovitch et al. [12] have shown that pH_{SS} in intertriginous regions of diabetic patients is significantly increased compared to nondiabetic subjects. Therefore, skin care products should be designed in order to preserve or restore the physiologically protective acid mantle [6, 11]. Skin care products for the elderly or diabetics should be developed as water-in-oil (W/O) formulations because of prolonged skin-hydrating effects [13]. The development of stable W/O lotions and creams is more challenging compared to oil-in-water (O/W) formulations, especially if the pH of the water phase has to be acidic. Therefore, excipients must be chosen carefully to guarantee stability of the formulation because emulsifiers often hydrolyze under acidic conditions, leading to a loss of function and a disagreeable smell.

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Topical α-hydroxy acid (e.g. glycolic acid)-containing O/W formulations are widely used in cosmetics and dermatology [14]. In a previous study, we showed that the application of a 10% glycolic acid-containing O/W emulsion with pH 4 reduced not only the pH_{SS} but also led to a significant decrease of the pH in deeper layers of the SC (pH_{SC}), very likely even affecting the stratum granulosum [15].

To prove that similar effects can be achieved with glycolic acid-containing W/O emulsion with pH 4, three independent clinical studies were performed to investigate efficacy and tolerability – a study on pH_{SS} and pH_{SC} in healthy subjects, a study on pH_{SS} in diabetics and a 4-week study assessing efficacy (pH_{SS}) and tolerability in elderly subjects.

Methods

pH and Skin Hydration Measurement

pH_{SS} and pH_{SC} were recorded using a standard hydrogen glass electrode (SI Analytics GmbH, Mainz, Germany). Values were recorded 1 min after application of the electrode. Skin hydration was measured with a Corneometer MPA 5 CPU.

Cosmeceutical Formulation

For the study in diabetic subjects, we used a W/O emulsion containing water, cetearyl isononanoate, dicaprylyl ether, cera alba, hexyl laurate, caprylic/capric triglyceride, glycerin, PEG-7 hydrogenated castor oil, cetyl alcohol, zinc stearate, ceresin, glycolic acid, phenoxyethanol, magnesium sulfate, PEG-30 dipolyhydroxystearate, ethyl linoleate, glyceryl caprylate, ethyl oleate, ethyl palmitate, tocopherol, ethyl stearate, *Helianthus annuus* seed oil, and allantoin. In all other studies, we used a W/O emulsion consisting of water, sorbitan oleate, polyglyceryl-3-polyricinoleate, isohexadecane, ethylhexyl stearate, decyl oleate, sucrose polystearate, tocopherol, ammonium hydroxide, glycerol, magnesium sulfate, and fragrance: limonene, linalool and citral. Glycolic acid was added to the water phase of the formulations to achieve a pH of about 4.

Study Subjects

The volunteers had not exercised, washed or applied topical formulations to the measured areas for at least 24 h prior to the measurements. All participants were provided with verbal as well as written information on the study and informed consent was obtained from each subject. All experiments were conducted in accordance with the current version of the Declaration of Helsinki.

Clinical Trial in Healthy Subjects to Assess pH_{SS} and pH_{SC}

None of the volunteers (n = 6, 29.9 ± 4.7 years) had a history of skin disorders nor did they suffer from a skin condition at the time of measurement. A pH 4 W/O emulsion (2 mg/cm^2) was applied homogenously on the volar forearm. Tape stripping was performed 10 min after application and pH_{SC} was measured after every 10 tape strippings. In total, 100 tape strippings were performed to remove the complete SC [16, 17]. Changes in pH_{SS} over time were measured without removal of the SC on the other arm.

Clinical Trial in Diabetics to Assess pH_{SS}

Male volunteers (diabetics: n = 10, 70.2 ± 2.6 years; nondiabetics: n = 10, 59.8 ± 3.5 years) were included for measurement of pH_{SS} of the axillary, inguinal, interdigital and plantar region, as well as of the lower leg and the dorsum of the foot. In addition, diabetic volunteers applied a pH 4 W/O emulsion to one foot of each patient (randomized) twice daily for 2 weeks and pH_{SS} was measured thereafter. The respective untreated foot of the other side served as control. All diabetic volunteers suffered from insulin-dependent diabetes mellitus. Diabetes was diagnosed 12.3 ± 2.9 years prior to measurements. HbA_{1c} of diabetics amounted to 7.8 ± 0.4% and blood sugar levels were 164 ± 18.1 and 131.4 ± 12.7 mg/dl, respectively (data not shown). Bacterial colonization was assessed by swabs before and after application of the emulsion. However, due to the limited sample size, there was only a trend towards bacterial reduction following application of the emulsion (data not shown).

Clinical Trial to Assess Long-Term Efficacy and Tolerability

A 28-day trial was performed (n = 30, 70.2 ± 5.2 years). The pH 4 W/O emulsion was applied 2–4 times daily by choice of volunteers on the volar forearm. The respective untreated volar forearm of the other arm served as control. Skin hydration and pH_{SS} were measured at baseline, after 14 days of application and finally at day 28. A subjective evaluation of the treatment was done by questionnaire.

Statistics

All data are presented as mean ± standard error of the mean (SEM). For statistical analyses, H^+ concentrations were calculated from the respective pH values. In terms of baseline pH_{SS}, differences between localizations were analyzed with one-way Kruskal-Wallis ANOVA on rank and post hoc Tukey tests. For comparisons of pH before and after treatment, paired t tests (in case normality testing passed) and Wilcoxon signed-rank tests (in case normality testing failed) were done. Long-term study was evaluated by descriptive statistics using the Wilcoxon signed-rank test.

Results

Clinical Trial in Healthy Subjects to Assess pH_{SS} and pH_{SC}

The overall impact of the pH 4 W/O emulsion was initially investigated on healthy subjects. Baseline pH_{SS} was measured at 4.44 ± 0.18. Immediately after application of the pH 4 O/W emulsion, pH_{SS} was 3.53 ± 0.13. pH_{SS} then increased steadily over the next 2 h, finally reaching 4.38 ± 0.32 (fig. 1a). The decline of pH_{SC} after the application of pH 4 W/O emulsion could also be measured in deeper layers of the SC. After 10 tape strippings, a pH_{SC} of 4.07 ±

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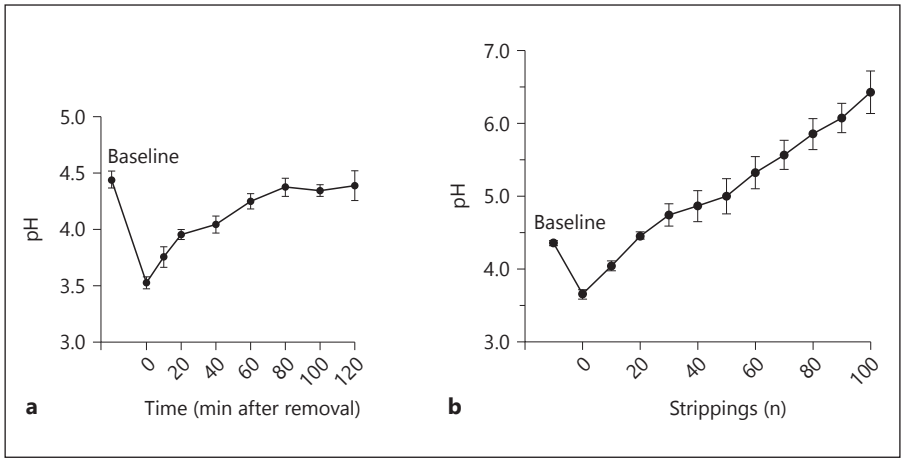

Fig. 1. The pH4 W/O emulsion (2 mg/cm^2) was applied homogenously on the volar forearm of healthy volunteers (n = 6, 29.9 ± 4.7 years). **a** pH_{SC} was recorded using a standard hydrogen glass electrode. **b** Tape stripping was performed 10 min after application and pH_{SC} was measured after every 10 tape strippings.

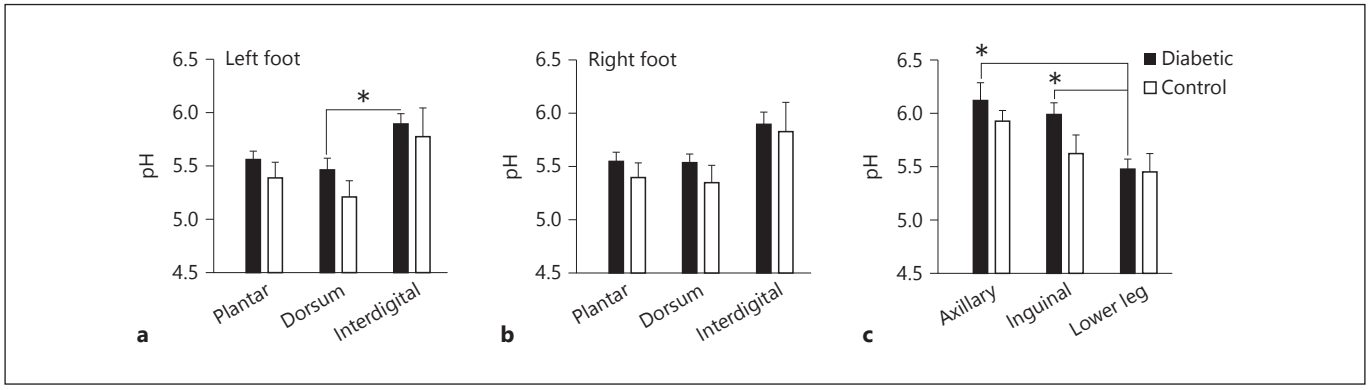

Fig. 2. Male volunteers (diabetics: n = 10, 70.2 ± 2.6 years; nondiabetics: n = 10, 59.8 ± 3.5 years) were included for measurement of pH_{SS} of plantar, dorsum and interdigital regions of the left foot (**a**), plantar, dorsum and interdigital regions of the right foot (**b**) and axillary and inguinal regions, as well as the lower leg (**c**). Mean ± SEM. * $p < 0.05$, post hoc Tukey test.

0.22 was measured and with further strippings pH_{SC} still remained markedly reduced (fig. 1b) compared to values we had previously measured on untreated skin [18].

Clinical Trial in Diabetics to Assess pH_{SS}

There were no significant differences in pH_{SS} between diabetics and controls on the bottom and the dorsum of the foot, as well as in the interdigital region (fig. 2a, b). Additionally, no significant differences were detected between the left and right foot – both for diabetics and controls. However, interdigital pH_{SS} of the left foot was significantly higher compared to pH_{SS} on the dorsum (fig. 2a). A similar tendency was seen on the right foot but the results were not significant (fig. 2b). For the other localizations (axillary and inguinal regions and the lower leg), there were no significant differences in pH_{SS} between diabetics and controls (fig. 2c). In diabetic patients, however, we found significantly lower pH_{SS} values on the lower leg compared to the axillary and inguinal regions. Even though not significant, pH_{SS} was slightly higher at all localizations in diabetics than in control patients (fig. 2a–c). Overall, intertriginous regions exhibited slightly higher pH_{SS} values. Topical application of pH 4 W/O emulsion for 2 weeks led to a significant reduction in pH_{SS} on the bottom and the dorsum of the foot, as well as interdigitally (fig. 3).

Clinical Trial to Assess Long-Term Efficacy and Tolerability

Evaluation of long-term efficacy and tolerability was addressed over 28 days in elderly volunteers. Already after 14 days but also after 28 days of treatment, a statistically significant increase in skin hydration was observed at the treated volar forearm in all study participants com-

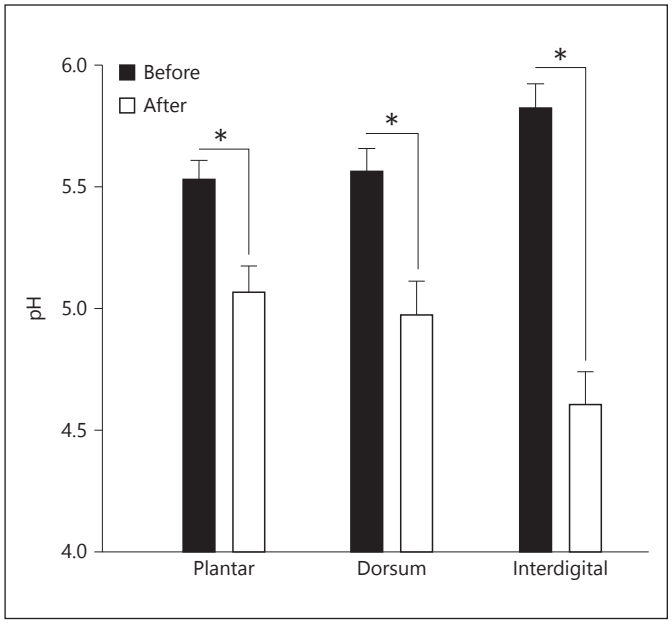

Fig. 3. In addition, diabetic volunteers applied a pH 4 W/O emulsion to one foot of each patient (randomized) twice daily for 2 weeks and pH_{SS} reduced significantly on the bottom of the foot ($p = 0.002$, Wilcoxon signed-rank test) and the dorsum of the foot ($p = 0.002$, Wilcoxon signed-rank test), as well as interdigitally ($p = 0.005$, paired t test). The respective untreated foot of the other side served as control.

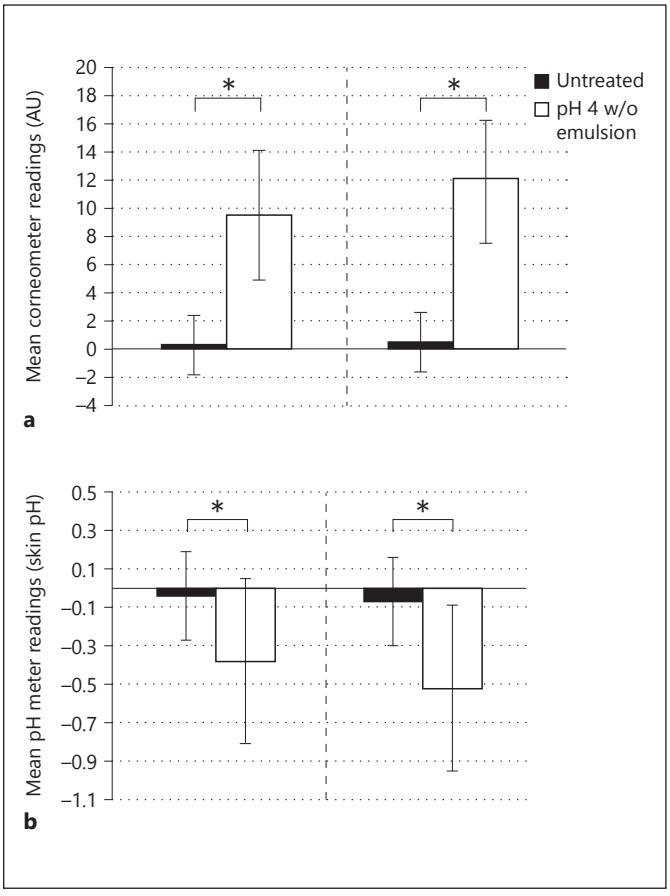

Fig. 4. A 28-day trial was performed (n = 30, 70.2 ± 5.2 years). A pH4 W/O emulsion was applied 2–4 times daily by choice of volunteers on the volar forearm. The respective untreated volar forearm of the other arm served as control. **a** Skin hydration was measured after 14 days of application and finally at day 28 (Δ-values calculated to baseline). **b** pH_{SS} was measured after 14 days of application and finally at day 28 (Δ-values calculated to baseline).

pared to the untreated control site. Mean Corneometer readings increased by 9.5 units after 14 days and increased further by 12.1 units after 28 days (fig. 4a). Additionally, pH_{SS} values were reduced by 0.38 after 14 days and even by 0.52 after 28 days of application compared to untreated areas on the respective other volar forearm (fig. 4b).

Discussion

In contrast to previous studies investigating a pH 4 O/W emulsion [15], the effect on pH_{SS} of a pH 4 W/O emulsion was investigated regarding the acidifying properties. The two different emulsion types (W/O and O/W) exhibit different properties and thus differ with respect to influencing the pH_{SS}. In the case of a W/O emulsion the acidified inner water phase must be released, which highly depends on the stability of the emulsion. A stable W/O emulsion would hypothetically slow down the release of H^+ and a delayed pH_{SS} declining effect compared to O/W emulsion can be observed. However, as shown in this short-term study in healthy volunteers, rather immediately after topical application of the pH 4 W/O emulsion a significant decrease of pH_{SS} could be measured. This rapid positive effect on pH_{SS} emphasizes the appropriate galenical formulation of the pH 4 W/O emulsion. Since no data are available, it is hypothesized that the speed of pH regulation by W/O emulsions depends strongly on the release of the inner acidic water phase. This can only be achieved by an intended release due to the adjusted stability of the W/O emulsion by the use of appropriate emulsifiers [U. Knie, pers. commun.].

Different factors (e.g. aging or diseases like diabetes mellitus) influence the pH_{SS} and therefore impact the physiological functions of the skin negatively. The difference of pH_{SS} in diabetics and control patients was not

significantly different in our study – maybe due to the limited number of subjects. In contrast, an increased pH_{SS} in diabetics was observed at all localizations, according to the literature [12]. However, the treatment of diabetic patients with a pH 4 W/O emulsion led to a significant reduction of pH_{SS} on the bottom of the foot, the dorsum of the foot and in the interdigital area of the foot, restoring a physiological pH_{SS}. A physiological pH_{SS} may lead to improved epidermal barrier integrity, thus perhaps reducing cutaneous manifestations, e.g. microbial infections, due to an impaired epidermal barrier in diabetic patients [1, 4]. Furthermore, it is known that also during aging, pH_{SS} increases at different localizations (forearm, temple and forehead) [2], which contributes to reduced epidermal integrity and impaired epidermal homeostasis. Furthermore, these changes render the skin more susceptible to microbial colonization [1, 3, 4, 19].

To the best of our knowledge, this is the first study using a pH 4-adjusted W/O emulsion to revert an increased skin pH in the elderly, as recommended by Maibach and Levin [11]. Topical long-term application of the pH 4 W/O emulsion resulted not only in increased skin hydration but also in a significant decrease of pH_{SS} already after 14 days. This effect continued until the end of the study (day 28) and a further decrease was observed. This finding is not in contrast to an independent study by Buraczewska and Lodén [20], where they failed to prove the superiority of a cream of pH 4.0 over a cream of pH 7.5 regarding the promotion of skin barrier recovery. However, application in this study was only for a short-term period of 7 days. Interestingly, comparable effects were observed with a pH 4 O/W emulsion after 14 days of application [21]. However, in this study no further decrease after 14 days was observed. This limited pH_{SS} adjusting effect of the pH 4 O/W emulsion is probably due to the different galenical formulation of the emulsion and an only once daily application compared to the study presented here with an application of W/O emulsion 2–4 times daily, which is very likely more effective.

In conclusion, the skin of elderly and diabetic subjects exhibiting a higher pH_{SS} is very likely to benefit from skin care products with an acidic pH to normalize increased pH_{SS} values. Regular application may improve skin functions. Moreover, acidifying the skin surface to physiological pH values reduces dry skin as well as skin sensitivity towards infection and irritation by improving cohesion of corneocytes and homeostasis of the epidermal barrier. The latter effect may depend upon acidic sphingomyelinase and β-glucocerebrosidase, which display a pH optimum below 5 [22]. Since no data are available, investigations of changes in the lipid composition of the epidermal barrier following the application of acidic formulations (pH 4) are needed to better understand the underlying mechanisms of the constitution and maintenance of a physiological and healthy epidermal barrier.

Disclosure Statement

P.B. received honoraria for conducting the above study from Dr. August Wolff Arzneimittel GmbH & Co. KG. The authors C.A. and M.K. are employees of Dr. August Wolff Arzneimittel GmbH & Co. KG.

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