Skin Pharmacology and Physiology

Skin Pharmacol Physiol , DOI: 10.1159/000522289
Received: July 26, 2021
Accepted: January 26, 2022
Published online: January 28, 2022

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ISSN: 1660-5527 (Print), eISSN: 1660-5535 (Online)
https://www.karger.com/SPP
Skin Pharmacology and Physiology

Research Article

An exploratory study of the effects of the pH of synthetic urine on skin integrity in healthy participants

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Short Title: Effects of synthetic urine on healthy skin

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Number of Tables: 0
Number of Figures: 5
Word count: 3077
Keywords: acid mantle; incontinence-associated dermatitis; skin barrier; skin health; synthetic urine

Abstract

Background: Incontinence-associated dermatitis (IAD) develops from prolonged exposure of skin to urine and/or stool and represents a common complication in older adults, reducing the quality of life. Increased pH is an important etiologic factor of IAD, however, the relationship between urinary pH and skin barrier disruption remains unclear.

Objective: To examine the effects of synthetic urine (s-urine) at various pH on transepidermal water loss (TEWL), stratum corneum hydration (SCH) and skin surface pH.

Methods: S-urine solutions (pH 5.0-9.0) were applied to the volar forearms of 15 healthy participants for 2 hrs, with another site serving as the untreated control. Measurements of TEWL, SCH and skin surface pH were obtained at baseline and after each challenge. Skin buffering capacity was also examined in 5 volunteers by recording skin pH at baseline, after 2 hrs exposure and every 5 mins for 40 mins.

Results: TEWL and SCH were increased following exposure to s-urine compared to baseline values. Although there was tendency for pH to an increase after exposure, further investigation showed that changes are only temporal as pH value is restored to baseline within 5 mins. There were no significant differences between solutions.

Conclusions: This study revealed that urine disrupts healthy skin integrity; however, its effects are not pH dependent. Transient changes were observed on the acid mantle of the skin due to its innate buffering capacity. Future studies need to examine the effects of urine combined with bacteria responsible for pH elevation in patients with urinary incontinence.

Introduction

Incontinence-associated dermatitis (IAD) is classified by the ICD-11 [1] as a form of irritant contact dermatitis caused by the prolonged exposure of the skin to urine, faeces or both, and represents a common complication in adults with incontinence [2,3]. The initial clinical signs of IAD include persistent erythema and inflammation at the skin surface, and if left untreated, can lead to oedema, swelling and blister formation [2]. Affected individuals may experience pain, discomfort, burning, and itching, that ultimately reduce the quality of life, and are also at increased risk of developing pressure injuries [4,5]. Prevalence and incidence of IAD vary depending on the care setting, with higher rates commonly seen in acute care settings (19-45.7%) [4,6–8] than in long-term care facilities (4.3-8.4%) [6,9,10] with corresponding incidence rates of 5.2-46.1% [4,6,8,9,11] and 3.4-25%, respectively [12–14].

The physical barrier of the skin resides in the stratum corneum (SC) with the important functions of cohesion, homeostasis, the regulation of water diffusion, a process associated with transepidermal water loss (TEWL) [15], and the protection of the body from various insults, involving microbes and UV light [16]. The acidic pH of the skin surface, known as the acid mantle [17], is a critical regulator of SC function [18] and any alterations in pH will impair the barrier, enhance the penetration of substances, inhibit normal skin microbiota, and promote the growth of pathogens [19–21]. By exhibiting a pH between 4 and 6, the skin surface provides an optimum environment for the activity of key enzymes involved in desquamation and ceramide synthesis, such as serine proteases and β-glucocerebrosidase, for the formation of lamellar bilayers, which are critical for skin permeability, and for the recovery rate of the barrier function in pathogenic conditions [21–27]. Therefore, TEWL, hydration of the SC (SCH), and skin's acidity are important physiological properties for the maintenance of skin integrity and consequently high values of these parameters are associated with an impaired barrier [15,28].

In incontinent patients, exposure to urine for extensive periods of time macerates the skin, leading to an overhydrated epidermis, swelling of corneocytes in the SC, barrier disruption and enhanced upregulation of pro-inflammatory cytokines [29–31]. The damage is aggravated in the presence of urease-producing bacteria from the perineum or the nearby urinary tract that convert urea in urine into ammonia. This converts skin pH to alkaline levels which can activate lipolytic and proteolytic enzymes from the gut and excreted in faeces [32–34]. Elevated surface pH, in turn, increases skin permeability thus irritating the effects of incontinence [35] and promoting microbial growth and IAD development [36,37]. Indeed, previous studies have reported that even short-term exposure to alkaline urine, results in erythema, with an associated increase in TEWL, SCH and skin pH, and an eventual compromise to skin barrier functionality [38,39]. In particular, an increase in urinary pH was associated with more severe disruption of barrier function [39]. However, previous studies investigating this, employed alkaline urinary values (pH 7.9-10.7), which are beyond those of biological urine ranging from pH 4.5 to pH 8.0 [40,41], depending on several factors, including diet and the presence of infection [40,42]. As a consequence, the exact relationship between urinary pH and disruption of skin integrity is still unknown. The present study examined the effects of synthetic urine (s-urine) at various pH values of physiological relevance on biophysical parameters characterizing healthy skin integrity.

Methods

Study design and setting

This study represents an exploratory study approved by the ethics committee of University of Southampton (approval number 9349) and conducted within a bioengineering laboratory under controlled temperature (22°C ± 3°C) and humidity (40-45%) conditions, as these influence measurements [43–47]. Although this did not represent a randomized control trial, we adhered to the CONSORT statement guidelines for the reporting of this study [48].

Participants

Previously published data using the measures of skin barrier function suggested that a change of 25% was detectable using 15 subjects with 80% power and significance at a significance level of 5% [49]. The inclusion criteria for recruiting participants were: 1) aged 18-65 years, 2) no active skin disease

and 3) no previous history of skin diseases. Exclusion criteria included pregnancy, pre-existing medical condition that is known to affect the dermal vasculature (e.g., diabetes mellitus), treatment with any vasoactive medication (e.g., beta-blockers, non-steroidal anti-inflammatory drugs; steroids), pre-existing dermatological condition and inability to give informed written consent. Participation was voluntary and no incentives were provided. Participants were asked to refrain from applying any cosmetic products to the forearms for 12 hrs before the study, to avoid influence on biophysical measurements [46,50]. Informed consent was obtained from the participants upon arrival to the laboratory and after the details of the procedures had been fully explained. All study procedures complied with the principles outlined in the Helsinki Declaration and participants were left to acclimatize to the ambient conditions for 30 mins prior to testing.

Synthetic human urine (S-urine)

To simulate the moisture irritant source experienced by patients with urinary incontinence, s-urine (pH of 7.9) was used as previously described [51]. In brief, 25g urea (Fisher Scientific, UK), 9g sodium chloride (Sigma Aldrich, UK), 3g ammonium chloride (Fisher Scientific, UK), 3g sodium sulphite (Fisher Scientific, UK), 2.5g anhydrous disodium hydrogen orthophosphate (Fisher Scientific, UK), and 2g creatinine (Across Organics, Geel, Belgium) were dissolved in 1L of distilled water and kept at 4°C. S-urine pH was adjusted to values from 5.0 and 9.0 with 1M hydrochloric acid and 1M ammonium hydroxide.

Skin integrity assessment

Skin integrity was evaluated using non-invasive biophysical measurement techniques. TEWL was quantified using the open-chamber Tewameter® TM 300 (Courage & Khazaka Electronic GmbH, Cologne, Germany), and a metal stand was used to hold the probe horizontally to maintain a constant applied pressure on the skin to reduce movement artefacts [52]. It is accepted that there is no optimum TEWL value for healthy skin and that there is considerable heterogeneity among studies. However, in one study using the open-chamber method, for individuals under 65 years old, low values of TEWL (≤10 g/h/m^2) were reported for the volar forearm [15]. By contrast, SCH values as determined using capacitance principles are generally characterized into three skin types depending on whether they are very dry i.e., <30 arbitrary units (AUs), dry 30-40 AUs or normal moist well-hydrated skin i.e., >40 AUs [53]. Typical skin surface pH has been reported to be within 4.5-5.0 in the forearm region, although variation is evident even across the same anatomical region [54]. All measurement probes were part of the Multiprobe Adapter MPA9 system (Courage & Khazaka Electronic GmbH, Cologne, Germany).

Study procedures

Participants attended the laboratory on two separate study visits, two weeks apart. All tests were performed on the volar aspect of both forearms as it represents an easily accessible site commonly used in dermatological research, thus facilitating comparison with other studies. At the main study visit, baseline measurements of TEWL and skin surface pH were taken on six areas (20mmx20mm, three in each forearm) at contralateral locations. Each test area was separated by a distance of 40 mm, determined using a ruler (Fig. 1a). Then, the different s-urine solutions were applied on five sites using HillTop chambers (25mm, HillTop Research Inc., Saint Petersburg, Florida), saturated with 500µl s-urine, and secured in place with transpore adhesive tape (3M, Minneapolis, Minnesota). The remaining site served as the untreated control, as illustrated in Figure 1b. The order of s-urine solutions was randomized among participants using a Latin square. After a 2-hour exposure period, the treatments were removed, and any excess moisture was removed by pat drying the skin with filter papers to ensure that what is measured is TEWL and not wet skin [55]. Then, biophysical measurements were repeated at all six sites (Fig. 1c). In the subsequent visit, baseline skin pH measurements were obtained on four areas in both forearms, and two acidic (pH 5.0 and pH 6.0) and one alkaline (pH 8.0) s-urine solutions were applied for 2 hrs on the skin, with the remaining site serving as the untreated control. Following that, treatments were removed, and the skin was pat dried, as previously. Skin surface pH was recorded immediately and then every 5 mins for a total period of 40 mins.

Data analysis

Each site served as its own control. For skin integrity parameters, data are expressed at baseline (BL) and after each challenge as median and interquartile ranges (IQR, 25th to 75th percentiles) and presented in box plots, created with GraphPad Prism 8 (GraphPad Software, San Diego, California, USA). Table 1 also summarizes median differences and the corresponding % change from baseline values. A line graph, showing medians with IQR, was also plotted to show changes in skin pH over time after the different challenges. All statistics were performed in SPSS version 25 (IBM Corporation, Armonk, NY, USA). Considering the small sample size (n=15), non-normal distribution of the results was assumed, and changes in biophysical measures from BL were determined by Wilcoxon signed-rank tests. Differences between s-urine solutions were assessed using the Friedman test followed by Wilcoxon signed-rank tests, respectively. A significance level of 5% i.e., $p < 0.05$ was considered statistically significant.

Results

Participants

The study was completed by 15 healthy participants (7 males, 8 females; mean age ± SD: 34.2 ± 12), who were recruited from the staff and student population of the University of Southampton. All volunteers participated in the main study, which explored the effects of s-urine of varying pH (pH 5.0-9.0) on the functional characteristics of skin integrity. Of those, five (mean age ± SD: 44 ± 12.30, 2 males, 3 females) participated in the subsequent study to investigate the temporal changes in skin pH when exposed to urine solutions of pH 5.0, 6.0 and 8.0.

Skin integrity

After exposure to s-urine solutions at different pH values, an increase in TEWL, SCH and skin surface pH compared to baseline values was revealed, as indicated in Table 1. For TEWL, these differences were statistically significant ($p<0.001$, in all cases, Fig. 2), with a median increase ranging from 25% to 40%. However, there was no significant difference in the degree of TEWL increase between the s-urine solutions at different pH values ($p=0.066$). In addition, there was a minimal change in TEWL at the control site, which was not statistically significant from baseline ($p=0.320$). For SCH (Fig. 3), the corresponding percentage increases from baseline were 8.9%, 6.1%, 16.2%, 6.7% and 13.4% for pH values of 5.0, 6.0,7.0,8.0, and 9.0, respectively. These differences were statistically significant for three of the pH solutions ($p=0.004$ for pH 5.0, $p=0.012$ for pH 6.0, $p=0.004$ for pH 7.0), but not for the solutions of pH 8.0 and 9.0 ($p>0.05$ in both cases). However, the differences between the effects of s-urine solutions were not statistically significant ($p=0.339$). In addition, there was a minimal change in skin hydration at the control site, which was not statistically significant from baseline ($p=0.865$). Skin surface pH (Fig. 4) was also shown to increase after exposure, with a median increase ranging from 2.5% to 5.7%, representing a statistically significant difference in all pH s-urine solutions ($p=0.001$ for pH 5.0, $p=0.002$ for pH 6.0, $p=0.008$ for pH 7.0, $p=0.002$ for pH 8.0, $p=0.004$ for pH 9.0). However, there was no significant difference in the degree of skin pH increase between the s-urine solutions at different pH values ($p=0.302$). It was interesting to note that the difference in skin pH at the control site was also found to be statistically significant ($p=0.038$).

Skin's buffering capacity

A transient rise in median skin pH from baseline was observed after exposure to s-urine solutions ranging from 0.55 to 0.61. However, pH value was restored back to baseline levels within five minutes and remained relatively constant by the end of measurements, as shown in Figure 5.

Discussion

This study examined the effects of s-urine solutions of different physiologically relevant pH (pH 5.0-9.0) on important biophysical characteristics of healthy skin integrity, namely TEWL, SCH and skin pH. Although it has been reported that prolonged exposure of the skin to urine disrupts skin barrier function, maceration, and elevates skin pH [37], the exact mechanism by which urine and its inherent pH contribute to IAD has not been reported so far.

Exposure to s-urine solutions for 2 hours caused an increase in TEWL and SCH, indicating skin barrier disruption and overhydration of the epidermis, although the effects were not pH-dependent. An increase in cutaneous pH was also evident, although these changes were found to be transient, as pH values were rapidly restored to baseline within five minutes, due to the inherent buffering capacity

of healthy skin. This is of clinical relevance as we previously demonstrated that damaged skin characterized by a compromised buffering capacity and disturbance of the acid mantle, as demonstrated with elderly adults [56], presents an increased permeability to irritants [35]. Taken together, these findings suggest that the use of pH-balanced cleansers should be used in skin care regimens to maintain the skin acid mantle or products to keep the amino acid pool of the epidermis constant, primarily responsible for the buffering capacity of the skin [57], may provide an effective prevention strategy for IAD. In particular, buffered skin care products and cleansers containing amino-acid surfactants, such as glycinates, sarcosinates and glutamates are superior and milder to the skin than the commonly used anionic sulphate surfactants, and can therefore be used as cleansing agents to maintain an optimal pH value [58–60]. Our findings are in contrast with a previous study which reported that an increase in urinary pH is associated with more severe disruption of barrier integrity [39]. This could be attributed to discrepancies in study designs with respect to exposure time and the degree of alkalinity of solutions. In particular, Larner and colleagues [39] employed alkaline urinary values (pH 7.9-10.7) while this study investigated a range of urinary values from pH 5.0-9.0 closely resembling the pH of biological urine.

It is currently widely accepted among clinicians that patients with urinary incontinence alone are less likely to develop IAD [4,6]. However, when combined with other factors skin damage, inflammation and IAD can occur. In particular, the presence of bacteria in urine is commonly found in patients with urinary incontinence [61] and indeed bacteria contaminated urine has been recognized as a risk factor for IAD [62]. Therefore future studies need to examine the mechanisms of IAD following combined exposure of skin to urine and common uropathogens associated with IAD, including *Pseudomonas aeruginosa* and *Proteus mirabilis*, which can also contribute to pH elevation due to its high urease activity [63].

Limitations

We acknowledge that this study was conducted on a relatively young cohort of healthy volunteers, and although age is not considered a risk factor of IAD, it is associated with a high prevalence of incontinence [64] and older adults are characterized by a diminished skin buffering capacity due to decreased barrier function [30,56]. Additionally, the prescribed 2-hour exposure period was practical for experimental testing on volunteers, however with the increasing use of absorbent pads to contain incontinence changing intervals can vary and urine may remain in contact with the skin for longer periods.

Conclusions

This study demonstrated that urine disrupts barrier function and overhydrates the epidermis, which are characteristics of macerated skin, but alone appears not to damage the protective acid mantle, which is critical in maintaining the integrity of the skin. Future work needs to examine the effects of urine combined with bacteria responsible for pH elevation in patients with urinary incontinence. This would further enhance our understanding of IAD development in patients with urinary incontinence.

Statements

Acknowledgement

The authors would like to express their gratitude to all the volunteers who participated in this study.

Statement of Ethics

This study was approved by the ethics committee of the Faculty of Health Sciences, University of Southampton (approval number 9349). Volunteers were recruited from the staff and student populations of the University of Southampton via word of mouth and a study poster. All participants provided written, informed consent prior to the start of the study and a copy of the consent form was given to each. No incentives for participation were offered. The study complied fully with the principles outlined in the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by a PhD studentship offered by the University of Southampton.

Author Contributions

All authors made substantial contributions to the conception and design of the study, SK performed acquisition of data, or analysis and interpretation of data; SK and DV involved in drafting the manuscript or revising it critically for important intellectual content; All authors gave final approval of the version to be published. SK agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Figure Legends

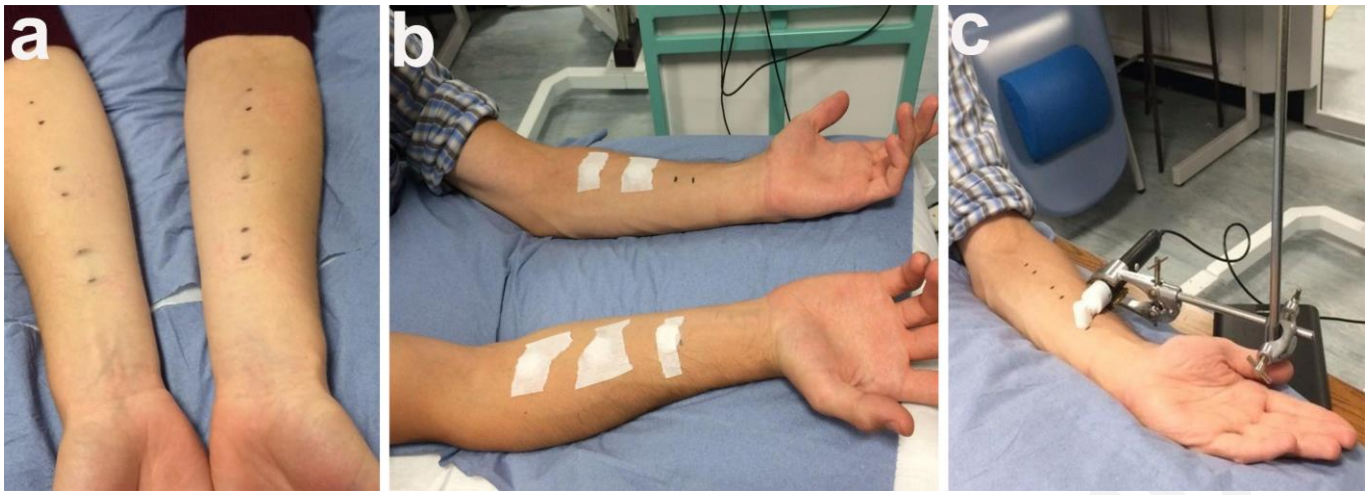
Fig. 1. Application of s-urine solutions. **a)** Six skin sites were marked on both volar forearms, and **b)** HillTop chambers were impregnated with 500µl s-urine and applied to the skin using a medical tape to keep them in place. A sixth site served as the untreated control. c) Prior to each treatment and after exposure, TEWL was measured to assess skin barrier disruption with the Tewameter® TM 300.

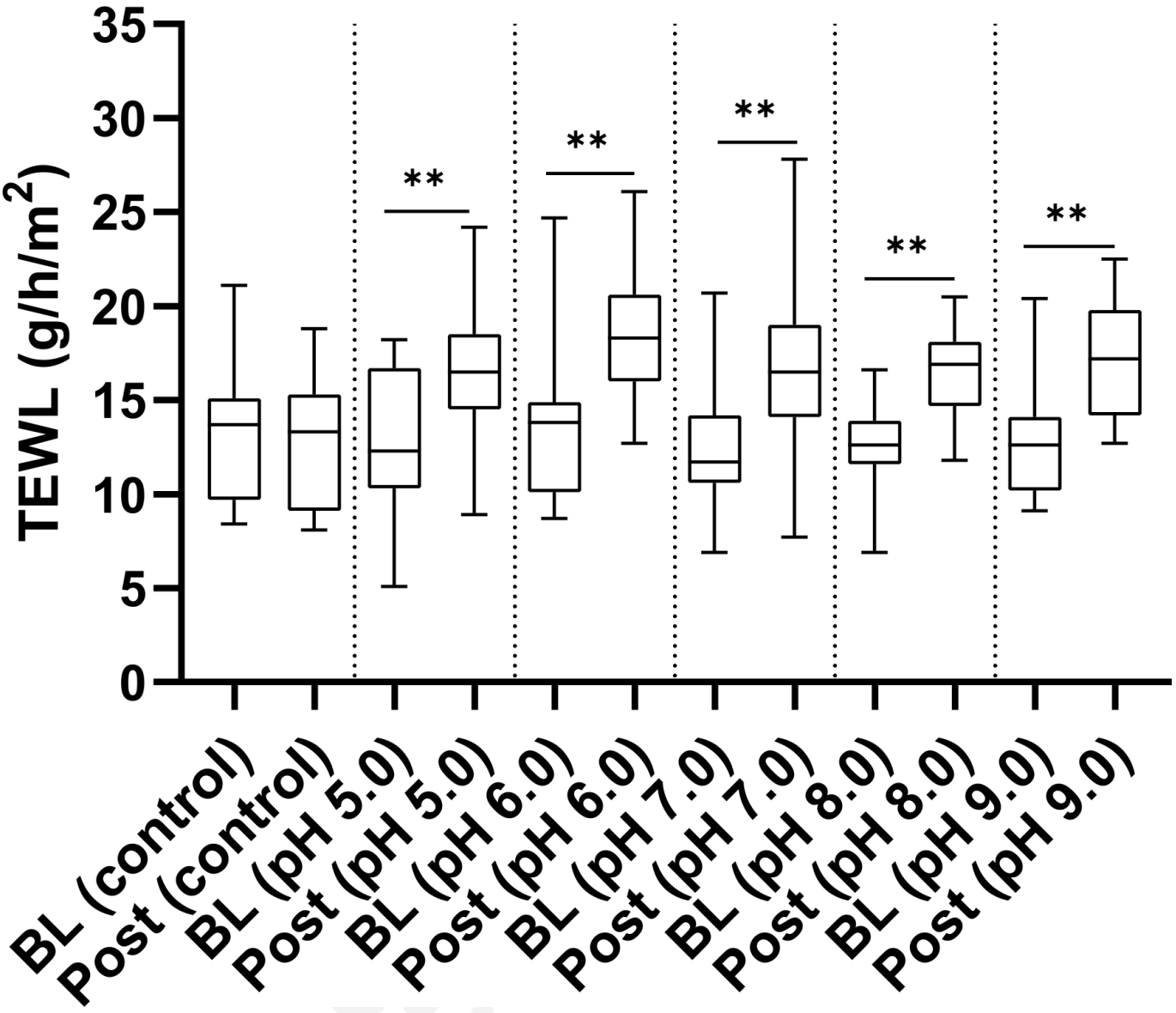
Fig. 2. Box and whisker plots for TEWL measurement at baseline and following exposure to s-urine solutions. All s-urine solutions caused a significant increase in TEWL compared to baseline values ($p<0.001$ in all cases). There were no significant differences between solutions ($p=0.066$).

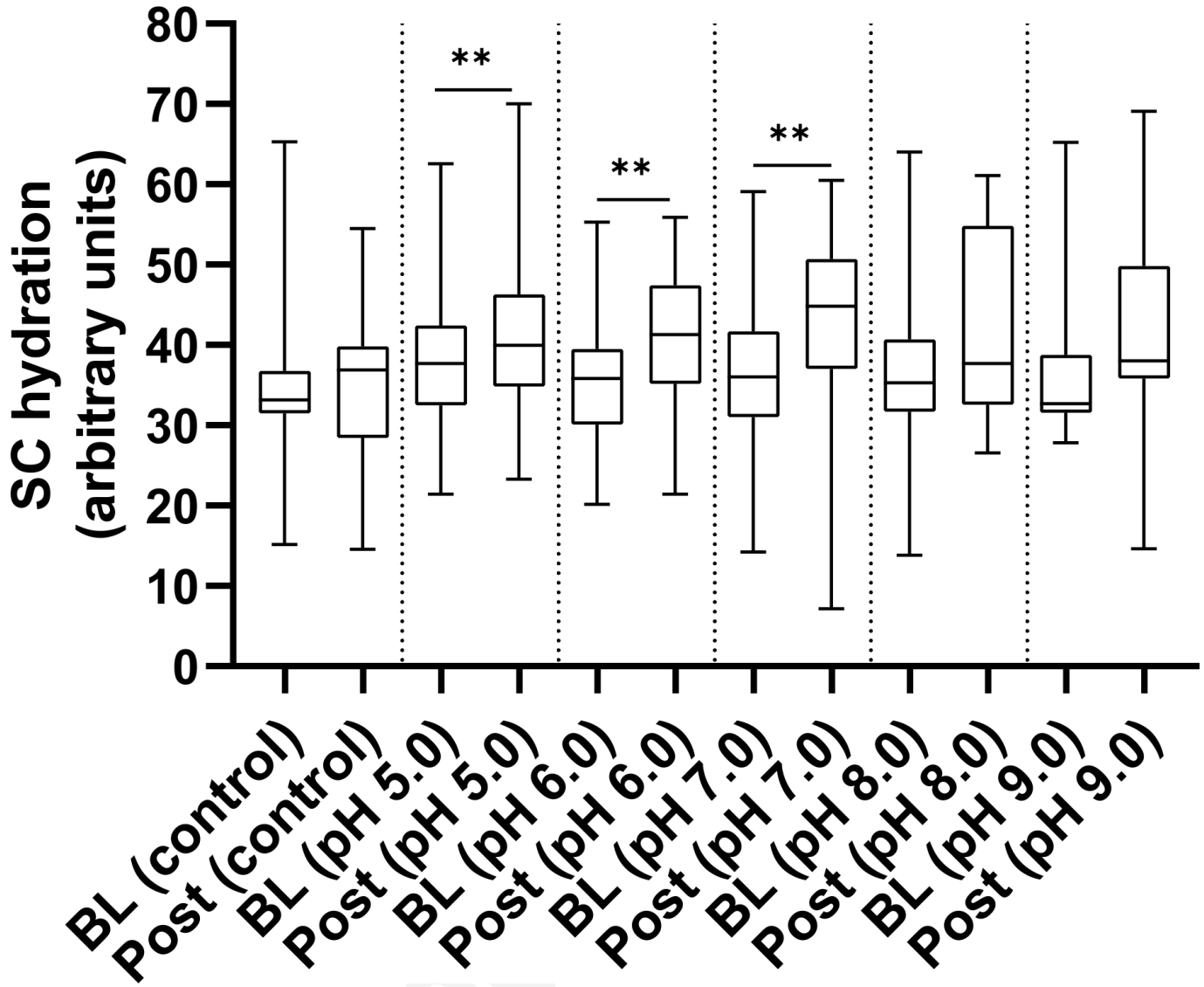
Fig. 3. Box and whisker plots for SCH at baseline and following exposure to s-urine solutions. Most s-urine solutions caused a significant increase in TEWL compared to baseline values ($p=0.004$ for pH 5.0, $p=0.012$ for pH 6.0, $p=0.004$ for pH 7.0. There were no significant differences for solutions with a pH of 8.0 and 9.0 ($p=0.069$ and $p=0.078$, respectively).

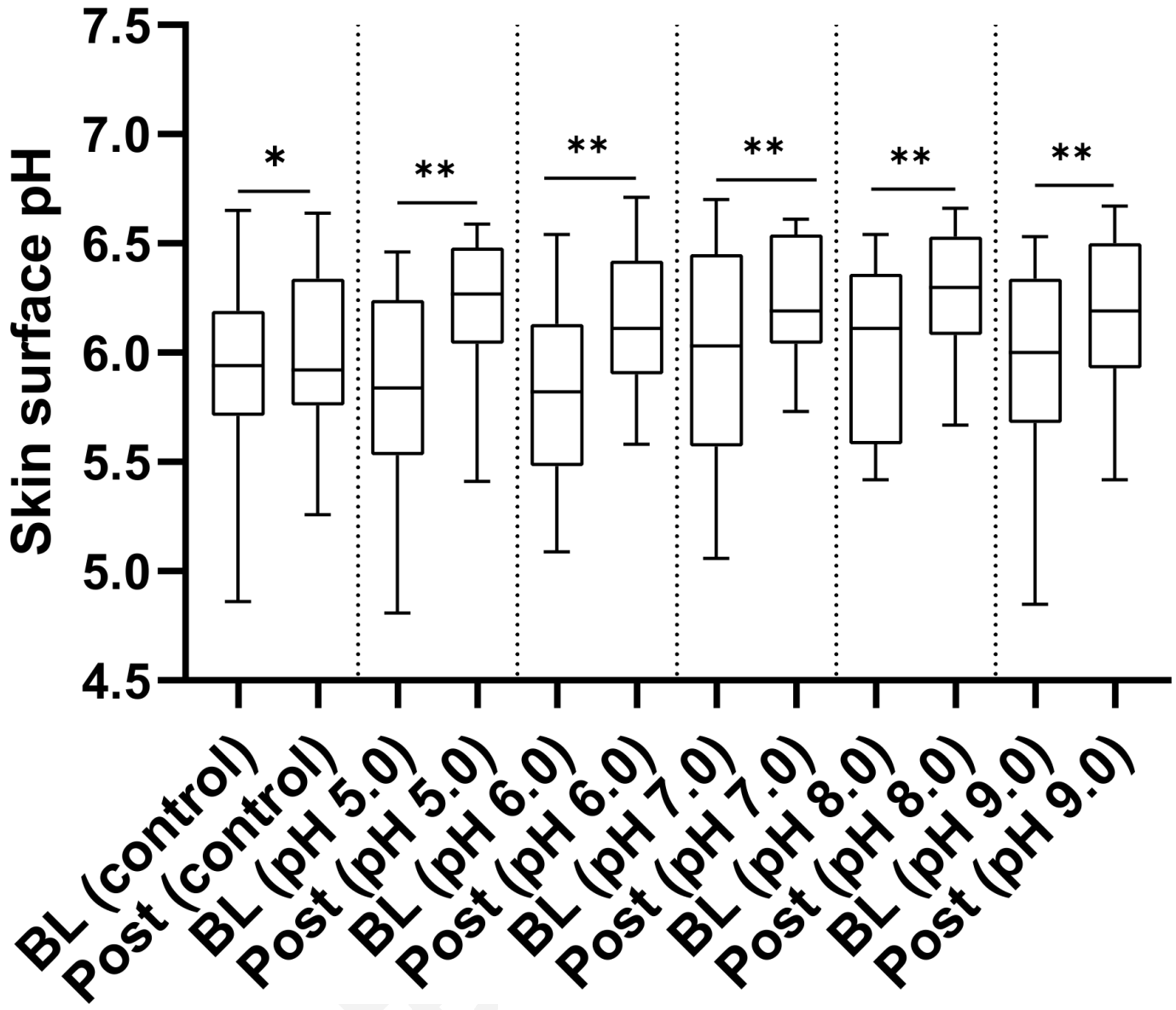
Fig. 4. Box and whisker plots for skin surface pH at baseline and following exposure to s-urine solutions. Significant increases in pH were observed at all skin sites, including the control ($p=0.038$ for control, $p=0.001$ for pH 5.0, $p=0.002$ for pH 6.0, $p=0.008$ for pH 7.0, $p=0.002$ for pH 8.0, $p=0.004$ for pH 9.0). No significant differences were found between the s-urine solutions ($p=0.302$).

Fig. 5. The buffering capacity of the skin following exposure to s-urine. The skin's buffering capacity was investigated after exposure to different s-urine solutions (pH 5.0, 6.0 and 8.0 ± 0.7). Note that after an initial increase in pH in all skin sites, skin pH returns to baseline values within 5 mins post-application.




TEWL (g/h/m2)
35
30
25
20
15
10
5
0
**
**
**
**
**
BL (control)
Post (control)
BL (pH 5.0)
Post (pH 5.0)
BL (pH 6.0)
Post (pH 6.0)
BL (pH 7.0)
Post (pH 7.0)
BL (pH 8.0)
Post (pH 8.0)
BL (pH 9.0)
Post (pH 9.0)


SC hydration
(arbitrary units)
80
70
60
50
40
30
20
10
0
**
**
**
BL (control)
Post (control)
BL (pH 5.0)
Post (pH 5.0)
BL (pH 6.0)
Post (pH 6.0)
BL (pH 7.0)
Post (pH 7.0)
BL (pH 8.0)
Post (pH 8.0)
BL (pH 9.0)
Post (pH 9.0)


Skin surface pH
7.5
7.0
6.5
6.0
5.5
5.0
4.5
*
**
**
**
**
**
BL (control)
Post (control)
BL (pH 5.0)
Post (pH 5.0)
BL (pH 6.0)
Post (pH 6.0)
BL (pH 7.0)
Post (pH 7.0)
BL (pH 8.0)
Post (pH 8.0)
BL (pH 9.0)
Post (pH 9.0)

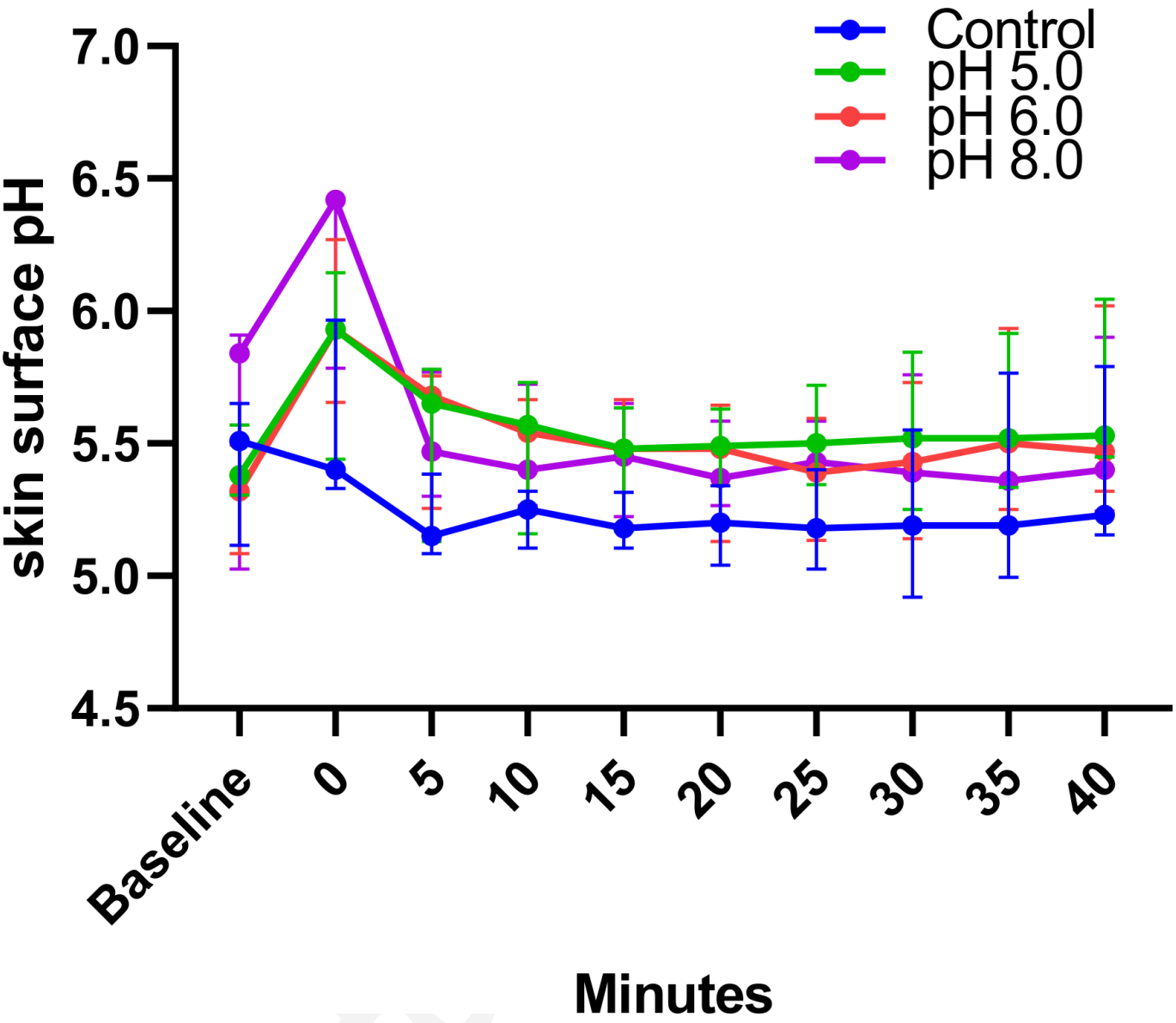

Control
pH 5.0
pH 6.0
pH 8.0
skin surface pH
7.0
6.5
6.0
5.5
5.0
4.5
Baseline
0
5
10
15
20
25
30
35
40
Minutes

Table 1. Median differences from baseline (BL) and % change for each skin integrity parameter following exposure to s-urine at different pH values.

	TEWL g/h/m²		SCH AUs		Skin surface pH pH units	
	Median difference	% change	Median difference	% change	Median difference	% change
Control	-0.40	-2.9 %	3.70	1.8 %	-0.02	2.1 %
pH 5.0	4.20	25.0 %	2.26	8.9 %	0.43	4.1 %
pH 6.0	4.50	38.4 %	5.46	6.1 %	0.29	5.7 %
pH 7.0	4.80	34.0 %	8.84	16.2 %	0.16	2.5 %
pH 8.0	4.30	25.9 %	2.36	6.7 %	0.19	3.8 %
pH 9.0	4.60	39.6 %	5.30	13.4 %	0.19	4.4 %